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CLINICAL AND EXPERIMENTAL

RESPIRATION OF MICROORGANISMS*†

F. G. NOVY, ANN ARBOR, MICH.

RESPIRATION is a fundamental characteristic of life. It is the source of energy which a cell must have in order to carry on its complex activities. The study of cell metabolism may be directed, and usually is, toward the kind and amount of intermediate, or of final cleavage products resulting from the utilization of carbohydrates, fats, proteins or other complexes. It may concern the action and nature of the diverse enzymes made by the organism, or it may deal with the physicochemical conditions within and without the cell which underlie all functional manifestations.

Every living cell respires whether it leads an independent existence or whether it is grouped with like cells to form the tissues and organs of higher plants or animals. The chief product is the gaseous CO_2 irrespective as to whether the given organism lives in the presence or absence of oxygen. To Lavoisier respiration was a combustion process in which molecular oxygen united with organic matter. It may still be so considered although the process itself is by no means as simple as this would imply.

For the study of the gas exchange or respiration of microorganisms two main procedures are available. In one a relatively large volume of gas is analyzed and for this purpose the gas buret in some form is used, preferably of the Henderson-Haldane type. In the other, which is essentially microchemical, the manometer of Barcroft is employed. The latter, of comparatively recent origin, has been used to determine the respiration of tissues as well as that of unicellular organisms such as yeast, bacteria, and protozoa.

The buret method is to be preferred whenever it is desired to study the respiration of a microorganism under varying conditions as regards the com-

*From the University of Michigan.
†George M. Kober Foundation Lecture, delivered at Gaston Hall, Georgetown University, Washington, D. C., March 28, 1931.

position of the medium and the influence of varying concentrations of O_2, CO_2 or other gases. In short it is applicable to the actual laboratory conditions under which a given culture is growing. The test tube culture is the one which is commonly used in the laboratory and for that reason it is highly desirable to have definite data as to the relative and absolute change that takes place within a tube.

The determination of the gaseous CO_2 in the air above the medium does not give the total amount of CO_2 formed. It is a well known fact that some organisms produce a considerable amount of basic products, and, if such is the case, some of the CO_2 will be fixed by the alkali. The estimation of this dissolved CO_2 requires a special procedure. The amount of CO_2 thus held in solution at times may be considerable. It may vary from practically zero to as much as 25 or 30 per cent of the total CO_2. The very low respiratory quotients often obtained with the Barcroft manometer and in other methods where the dissolved CO_2 is neglected may thus, in part, be accounted for.

A complete analysis, which includes the estimation of dissolved CO_2, involves the destruction of the organisms and for that reason it is not possible to have a count of the number present in the culture. A very rough approximation of that number could be made by counting the number present in a control culture grown under like conditions. Similarly, it would be possible to obtain the weight of the organisms in a control tube. Neither procedure was attempted.

For the reason given it was not possible to determine the extent to which the glycerine, glucose or other constituents of the medium were utilized during the respiratory period. The data sought and obtained represent therefore merely the gas exchange effected by the growth of a given organism on a fairly uniform surface area (15 to 18 sq. cm.).

The methods employed in this investigation have been fully described elsewhere.* It was necessary for this study to devise a manometer which would indicate the pressure changes taking place in the culture tube or jar. When it is desired to make an analysis, the manometer with its attached tube or jar is placed on a suitable platform and a connection is established with the gas buret. After displacement of the air in the connecting tube, with mercury, the gas in the culture tube is drawn into the buret and analyzed.

When this analysis has been made, the culture tube is taken out and aerated after which the dissolved CO_2 is determined. The sum of the gaseous and dissolved CO_2 represents the total CO_2 produced in a given experiment.

Carbonic Acid Production.—The amount and rate of production of this gas, under constant temperature conditions, varies with the organism and with the composition of the medium. Given the presence of a readily utilizable substance it is to be expected that multiplication of the organisms will be increased, and hence, an increased rate and intensity of the respiratory process.

Some data for the human tubercle bacillus are given in Table I. This organism does not grow on plain nutrient agar, and hence, the gas exchange is nil. On serum agar there is a fair growth in twenty-eight days with the

*Novy, F. G., Roehm, H. R., and Soule, M. H.: J. Infect. Dis. **36**: 109-167, 1925.

TABLE I

GAS EXCHANGE PER TUBE OF HUMAN TUBERCLE BACILLUS GROWN AT 37° C.
C.C. AT 0°, 760 MM.

	NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 37° C.
Plain Agar		0	0	
Glycerol Agar	12	48	57	334
	27	124	149	874
Glucose Agar	26	26	25	149
	85	66	70	410
Serum Agar	28	21	23	135

production of 21 c.c. of CO_2. The organism in this case is utilizing protein matter. When glucose is added to the agar, the organism avails itself of the carbohydrate with the result that more CO_2 is produced than on the serum agar and the growth itself is better.

The addition of glycerine to agar or potato is known to be beneficial in promoting the growth of the tubercle bacillus. It was originally employed with the view of preventing the desiccation of the medium during the long period of incubation. As a matter of fact, revealed by gas analysis, the advantage derived from glycerine lies in its ready utilization by the organism as a source of energy. It will be seen that the tubercle bacillus growing on this medium produced 124 c.c. of CO_2 in twenty-seven days, or 5 to 6 times as much as on glucose or serum agar.

For comparison, the gas exchange of the bovine tubercle bacillus is given in Table II. Here it will be seen that this organism grows about equally well on plain agar and on serum agar. The amount of CO_2 produced at the end of twenty-eight and fifty-six days is approximately the same for the two mediums. By contrast, with the human tubercle bacillus the bovine strain on the glucose medium gave, in twenty-eight days, a much higher yield of CO_2, 104 c.c. as against 26 c.c. Noteworthy is the fact that this represents the maximum development for on further incubation only a slight increase in CO_2 resulted (108 c.c.). The dissolved CO_2 at this point amounted to 7 c.c.,

TABLE II

GAS EXCHANGE PER TUBE OF BOVINE TUBERCLE BACILLUS GROWN AT 37° C.
C.C. AT 0°, 760 MM.

	NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 37° C.
Plain Agar	28	21	22	118
	56	24	29	170
Glycerol Agar	28	89	100	586
	56	155	174	1020
Glucose Agar	28	104	98	579
	56	108	105	616
Serum Agar	28	19	22	118
	56	28	33	193

showing the presence of an appreciable amount of alkali. Other oxidation products, undoubtedly, contributed to bring about cessation of growth. On glycerine agar, although at the end of twenty-eight days the production of CO_2 was somewhat less than on glucose agar, no inhibition of respiration took place on further incubation, and consequently at the end of fifty-six days, the culture produced 155 c.c. of CO_2 as compared with 108 c.c. on the glucose medium. As in the case of the human tubercle bacillus, the glycerine agar gave the best growths and the maximum gas exchange.

Table III presents a summary of the results obtained with the diphtheria bacillus. The CO_2 production at the end of seven and twenty-eight days was distinctly higher with glycerine agar than with the other mediums. Even more striking is the fact that while the production of CO_2 on the plain and

TABLE III

GAS EXCHANGE PER TUBE OF DIPHTHERIA BACILLUS GROWN AT 37° C. C.C. AT 0°, 760 MM.

	NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 37° C.
Plain Agar	7	16	17	99
	28	19	22	129
Glycerol Agar	7	21	26	152
	21	27	33	193
Glucose Agar	7	6	7	41
	28	20	23	134
Blood agar	7	18	19	111
	28	20	22	129

on serum agar, at the end of twenty-eight days, was about the same as that of the bovine tubercle bacillus, on glycerine and on glucose agar it was greatly inferior. Thus, on glucose agar only 20 c.c. of CO_2 were produced, while the bovine tubercle bacillus produced 108 c.c. The maximum growth of the diphtheria bacillus is attained in a shorter time, and hence respiration is retarded early. Compared with the tubercle bacillus a relatively small amount of energy is needed. To what extent inhibiting substances play a part in this cessation of growth is not known.

TABLE IV

GAS EXCHANGE PER TUBE OF GLANDERS BACILLUS GROWN AT 37° C. C.C. AT 0°, 760 MM.

	NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 37° C.
Plain Agar	7	17	21	123
	21	21	25	146
Glycerol Agar	7	88	102	598
	21	141	164	962
Glucose Agar	7	54	55	322
	21	122	126	739
Blood agar	7	38	45	264
	21	38	44	258

The results obtained with the glanders bacillus are given in Table IV. This particular strain grew fairly well on plain agar, and somewhat better on blood agar. On blood agar the maximum CO_2 production was obtained in about seven days (38 c.c.) since there was no further increase at the end of twenty-one days. On glucose agar the CO_2 production rose from 54 c.c. in seven days to 122 c.c. in twenty-one days. With glycerine agar the gas exchange was even more pronounced for the yield of CO_2 was 88 c.c. at the end of seven days and 141 c.c. in twenty-one days. The utilization of glucose and of glycerine here was more pronounced than in the case of the tubercle bacillus.

The results obtained with a nonpathogenic organism, the hay bacillus, are given in Table V. It might be expected that a saprophyte would be able to utilize freely proteins as well as carbohydrates. This is actually the case, since

TABLE V

GAS EXCHANGE PER TUBE OF HAY BACILLUS GROWN AT 32° C. C.C. AT 0°, 760 MM.

	NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 32° C.
Plain Agar	7	66	72	409
	56	84	85	483
Glycerol Agar	7	76	89	505
	20	205	244	1386
Glucose Agar	7	74	62	352
Serum Agar	7	65	75	426
	21	142	159	903

in seven days the yield of CO_2 on the four mediums was 65 to 76 c.c. which is considerably higher than the yield obtained with the other organisms mentioned, with the one exception of the glanders bacillus on glycerine agar. At the end of twenty days, the glycerine agar culture of the hay bacillus yielded 205 c.c. of CO_2 which is an unusual value.

The results obtained with the five bacterial organisms could be extended by the addition of others. They serve, however, to show that these organisms carry on an active respiration and yield a considerable though variable amount of CO_2. In this respect they differ in nowise from typical animal organisms, the protozoa. In Table VI will be found some of the results obtained with Trypanosome, Leishmania and Herpetomonas cultures. These organisms were grown on glucose blood agar and for that reason the values obtained are not directly comparable with those given in the preceding tables. It will be seen, however, that with one exception, these protozoa produced 60 to 70 or more c.c. of CO_2 in twenty-one to twenty-eight days. The slight irregularity may in part be due to uneven inoculation of the surface of the medium. This yield of CO_2 was greater than that obtained with the human tubercle bacillus, and the diphtheria bacillus on glucose agar.

That the unicellular bacteria and protozoa respire is evident from the data presented. In this respect they behave exactly the same as the cells in the complex animal body. Plant tissues, however, are no exception to the

TABLE VI

GAS EXCHANGE PER TUBE OF SOME PROTOZOA GROWN IN JARS AT 34° C. C.C. AT 0° C., 760 MM., MOIST; GLUCOSE BLOOD AGAR

ORGANISM	NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 34° C.
Tr. Lewisi	21	59	63	352
L. tropica	21	59	63	352
L. Donovani	28	62	65	363
L. infantum	28	29	29	162
H. oncopelti	28	72	75	419
H. culicidarum*	28	76	78	435
H. culicidarum**	28	73	76	424
H. lygaeorum	28	58	61	340
H. media	28	60	63	352
H. muscidarum	28	62	64	357
H. parva	28	58	62	345

*From Culex pipens.
**From Anopheles quadrimaculatus.

rule as has been shown by many investigators. As an example may be mentioned the respiratory change carried on by the potato. The results of two experiments are given in Table VII. In these tests strictly sterile cylinders of

TABLE VII

GAS EXCHANGE PER 10 GRAMS OF RAW POTATO AT 34° C. C.C. AT 0°, 760 MM.

NO. DAYS	CO_2 MADE	O_2 LOST	CORRESPONDING AIR VOLUME AT 34° C.
3	70	69	396
10	94	96	552

raw potato were placed in tubes which were connected with manometers in the same way as the in case of cultures. Analyses made at the end of three and ten days gave 70 and 94 c.c. of CO_2, respectively, calculated per 10 grams of potato. It may be added that as long as oxygen is present the potato respires the same as an aerobic organism. When the oxygen has been consumed it continues to respire but as an anaerobe.

Oxygen Consumption.—The production of CO_2 by a cell implies the utilization of oxygen regardless of whether that element is derived from the air or from compounds rich in oxygen. In the case of the aerobic organism it is the free oxygen of the air which is being consumed.

A cursory inspection of Tables I to VI will show that, as a rule, the volume of oxygen lost or rather consumed is somewhat larger than that of the CO_2

produced. The difference between the two volumes does not indicate that assimilation or retention of oxygen by the organism has taken place. It merely means that in addition to oxidizing carbon the oxygen has been used to burn hydrogen to water. It might even be used to oxidize the nitrogen or sulphur present in the protein which is being consumed. Oxygen consumption in excess of CO_2 production occurs when protein or glycerine is being utilized.

In the oxidation of a carbohydrate, such as glucose, $C_6H_{12}O_6$, the oxygen consumption should equal the CO_2 production. The values found when the organisms are grown on glucose agar or glucose blood closely approximate theory. They are usually a trifle higher and this indicates that the organism is chiefly oxidizing glucose and only to a slight extent protein matter.

It is noteworthy that the largest consumption of oxygen occurs when these organisms are grown on glycerine agar. This is to be expected considering the large amount of CO_2 produced. It merely means that glycerine is more readily utilized as a source of energy than are the carbohydrate or protein constituents of the medium.

The extent to which a given substance, such as glycerine, is utilized varies with different organisms. This fact can be made use of in differentiating between closely allied species. Thus, the oxygen consumption of B. melitensis when grown on glycerine agar is about two-thirds that of bovine B. abortus and less than one-half that of the porcine strain of B. abortus. A somewhat similar difference in the oxygen consumption of these three organisms was observed when they were grown on glucose agar.

Air Volume.—Without analytical data no inference can be drawn as to the volume of air which must be supplied to an organism in order to obtain a good growth. In general it has been supposed that there was enough oxygen in the air of a culture tube to meet its needs. The failure to realize that some organisms require a very large volume of air has led to false conclusions. Referring to Table I it will be seen that the tubercle bacillus at the end of twelve days, at which time only a slight growth had formed on glycerine agar had consumed 57 c.c. of O_2 which, at 37° C., corresponds to 334 c.c. of air. Obviously, no growth of this organism would be obtained in a sealed or stoppered tube of 20 c.c. capacity. To obtain a rich growth of this organism such as that given in twenty-seven days a volume of air corresponding to 874 c.c. must be made available. The relatively poorer growths on serum or glucose agar exhausted the oxygen from 135 and 149 c.c. of air. It is evident therefore that in the cultivation of the tubercle bacillus, whether it be the human or the bovine strain, a ready and free access of air must be provided. A pinhole through the sealing wax or paraffin used to seal the tube is sufficient for this purpose.

The relatively low consumption of O_2 by the diphtheria bacillus (Table III) is reflected in the corresponding volume of air. Thus, on glycerine agar, at the end of twenty-one days the organism exhausted the oxygen from 193 c.c. of air, which is a larger volume than was drawn upon by the culture on the other mediums. On the other hand the glanders bacillus in the same time utilized the oxygen out of 962 c.c. of air.

The largest consumption of oxygen was noted in connection with the hay bacillus (Table V) when grown on glycerine agar. The 244 c.c. of O_2 lost corresponds to an air volume of 1386 c.c. at 32° C. The avidity of this organism for air is in keeping with its saprophytic nature.

The protozoa, as shown in Table VI, when grown on glucose blood agar consume a fairly large amount of oxygen, 61 to 78 c.c., which corresponds to an air volume of 340 to 435 c.c. This is three times as much as the air volume utilized by the diphtheria bacillus when grown on glucose agar.

Respiratory Quotient.—This value is obtained by dividing the total volume of CO_2 produced by that of the O_2 consumed. The quotients calculated from the analytical data given in Tables I to V are assembled for convenience in Table VIII. It should be mentioned that in the tables the decimals of a cubic

TABLE VIII
RESPIRATORY QUOTIENTS FOR TABLES I TO V

	GLYCERINE AGAR	GLUCOSE AGAR	PLAIN AGAR	SERUM AGAR
Human Tubercle B.	(12) 0.83 (27) 0.84	(26) 1.04 (85) 0.94	. . . .	. . . . (28) 0.91
Bovine Tubercle B.	(28) 0.89 (56) 0.89	(28) 1.06 (56) 1.03	(28) 0.95 (56) 0.83	(28) 0.86 (56) 0.85
Diphtheria B.	(7) 0.81 (21) 0.82	(7) 0.86 (28) 0.87	(7) 0.94 (28) 0.86	(7) 0.94 (28) 0.91
Glanders B.	(7) 0.86 (21) 0.86	(7) 0.98 (21) 0.97	(7) 0.81 (21) 0.84	(7) 0.84 (21) 0.86
Hay B.	(7) 0.85 (20) 0.84	(7) 1.19	(7) 0.91 (86) 0.88	(7) 0.86 (21) 0.89

*The figures in parenthesis indicate the age of the culture in days.

centimeter were omitted and consequently the quotients given in Table VIII are only approximate. They are, however, sufficiently accurate for the purpose of comparison.

If an organism growing on a given medium such as glycerine agar, consumed only glycerine then the respiratory quotient should correspond to the theoretical value or 0.857. Similarly, with glucose agar the value would be 1.0. It is obvious, however, that an organism while utilizing such substances as a source of energy must also make use of other nutrient compounds such as proteins and amino acids. The result is that the observed quotient may be somewhat higher or lower than the expected theoretical value.

It will be seen from Table VIII that the quotients for the five organisms, grown on glycerine agar, approximate the theoretical value of 0.85. Similarly, the quotients for the cultures grown on glucose agar approach the value of 1.0. The most marked discrepancy is that presented by the diphtheria bacillus and this would indicate that it makes relatively very little use of glucose. The gas exchange (Table III) due to this organism whether grown on plain agar, glucose agar, or blood agar is much the same.

The quotients obtained for the cultures on plain agar and on serum agar are appreciably higher than the theoretical quotient for protein. It must be

assumed therefore that other substances having a higher quotient than 0.81 are undergoing oxidation.

Deserving of note is the fact that young and old cultures of a given organism have essentially the same respiratory quotient. The ratio of gas exchange, as might be expected, is the same for a small number of young cells as for the greatly increased number represented by an old culture. Conceivably, with old cultures decarboxylation could be expected, as a secondary reaction, in which case the quotient would be higher than at the beginning.

In Tables IX, X and XI are given average values for the respiratory quotients of these organisms when grown on the different mediums. The number

TABLE IX

AVERAGE OF CORRECTED REAL RESPIRATORY QUOTIENTS OBTAINED IN JAR EXPERIMENTS*

MEDIUM	THEORY	TUBERCLE BACILLI	
		HUMAN	BOVINE
Plain agar	0.81	. . . .	0.888 (4)
Glycerol agar	0.857	0.856 (13)	0.903 (9)
Glucose agar	1.0	0.992 (3)	1.036 (3)
Serum agar	0.81	0.904 (3)	0.852 (4)

*The figures in parenthesis give the number of experiments.

TABLE X

AVERAGE OF CORRECTED REAL RESPIRATORY QUOTIENTS OBTAINED IN JAR EXPERIMENTS*

MEDIUM	THEORY	DIPHTHERIA B.	GLANDERS B.	HAY B.
Plain agar	0.81	0.921 (4)	0.841 (5)	0.912 (10)
Glycerol agar	0.857	0.802 (4)	0.859 (4)	0.843 (8)
Glucose agar	1.0	0.906 (4)	0.972 (4)	1.278 (9)
Serum agar	0.81	0.942 (4)†	0.848 (4)†	0.874 (8)
Potato	1.0	. . . .	1.044 (4)	. . . .

*The figures in parenthesis give the number of experiments.
†Blood agar used.

TABLE XI

AVERAGE OF CORRECTED REAL RESPIRATORY QUOTIENTS OBTAINED IN JAR EXPERIMENTS. PROTOZOA AND POTATO*

MEDIUM	TR. LEWISI	L. TROPICA	L. INFANTUM	L. DONOVANI
Blood agar	0.822 (4)	0.875 (4)	0.868 (4)	0.862 (4)
Glycerol Blood agar	. . . .	. . . .	0.791 (2)	0.832 (2)
Glucose Blood agar	0.938 (3)	0.951 (3)	1.002 (4)	0.968 (4)
R. Q. of Potato:	Theory 1.0; found 1.006 (3)			

*The figures in parenthesis give the number of experiments.

in parenthesis following each quotient indicates the number of experiments made and averaged. These average respiratory quotients can be considered as fairly accurate values for the given organisms when grown on the stated mediums.

Varying Oxygen Concentrations.—When an agar tube inoculated with an aerobic organism is placed in a small jar which is attached to a manometer it will be evident before long that growth and respiratory changes are taking place. The growth continues until the manometer shows a constant reading at which point the oxygen content of the confined air volume is reduced to zero. It follows therefore that the organism, starting with 20.9 per cent of oxygen, has been growing in progressively decreasing concentrations of that gas. An experiment such as this does not reveal the effect, if any, of varying tensions of oxygen on the inoculum itself. This can only be ascertained by placing each one of several tubes in a separate jar into which a definite volume of oxygen is introduced. An essential condition for the success of a comparative experiment of this kind is that the absolute volume of oxygen necessary for good growth shall be practically the same in each container. This requirement will necessitate increasing the size of the container with each decreasing tension. It has been shown that to obtain a good growth of the tubercle bacillus, about 100 c.c. of oxygen must be made available. If, therefore, it is desired to have concentrations of oxygen corresponding to 5, 3, 1, and 0.5 per cent, it will be necessary to use containers of 2, 3.3, 10, and 20 liters capacity to supply the requisite 100 c.c. of oxygen.

Experiments made with the tubercle bacillus under conditions approximating those just given revealed the interesting fact that the growth of the organism was not proportional to the amount of O_2 consumed. Since each container held about 100 c.c. of O_2 and all of this was consumed, it could be expected that the growth would be quantitatively alike. Such was not the case; the growth mass decreased progressively with the decrease in the initial O_2 tension. In the lowest tensions there was lessened multiplication and slower removal of O_2. The final growth mass in the bottle which originally had 0.5 per cent O_2, after all the O_2 was consumed, was greatly inferior to that in the tubes which had the initial tensions of 3 or 5 per cent O_2.

This result appears to be of considerable significance. In the tissues the O_2 tension is low and consequently the multiplication of the tubercle bacillus is slow. Any measure which would tend to lower the tension would decrease still further the rate of multiplication of the organisms, and thus favor recovery of the individual. In tuberculosis, elimination of physical exertion by complete rest in bed and abundant feeding conceivably owe their beneficial results to a lowering of the O_2 tension in the diseased tissue. Complete removal of O_2 would cause the eventual death of the tubercle bacillus.

Although oxygen is essential to aerobic life yet in concentrations higher than that of air, it may be injurious. Lavoisier was the first to point out the deleterious effect of pure oxygen on animals and this fact has been repeatedly confirmed. Of the unicellular organisms, protozoa appear to be particularly susceptible to high oxygen concentrations. Thus, the development of cultures

of Trypanosoma lewisi is distinctly inhibited by a concentration of 45 per cent O_2 or about twice that in ordinary air. Cultures of Leishmania were found to be less sensitive, since they grew perfectly well in 50 per cent O_2 but with 60 per cent oxygen some evidence of inhibition was noted. This was more marked with 80 per cent and was complete with 100 per cent. Certain parasitic and free living protozoa when exposed to pure oxygen under high pressure were found by Cleveland to be actually killed.

Like the protozoa, bacteria show a varying response on exposure to high tensions of oxygen. In general, however, they are considerably more resistant. The glanders bacillus showed no difference in growth or gas exchange when grown in air and in 99 per cent oxygen. The human and bovine strains of the tubercle bacillus were apparently stimulated by tensions of 40 to 60 per cent. Good growth was obtained in 80 and 100 per cent oxygen, but it was not evenly distributed over the surface. Instead, isolated heaped-up colonies developed. The organisms outside of these colonies were killed. Apparently, the bacilli in the small masses of the inoculated material were protected against the injurious effect and were able to multiply whereas the isolated bacteria were destroyed. It is possible that a perfectly distributed inoculum would be completely inhibited in its growth and even destroyed by exposure to 100 per cent O_2. All told about six bacteria have been found by others to be inhibited by high concentrations of oxygen, but these results are open to the objection that soda-lime was used in the containers. The removal of CO_2 is in itself an inhibiting factor.

Varying CO_2 Concentrations.—When an organism is grown in a limited air volume, the oxygen is completely consumed and is replaced by 17 to 21 per cent of CO_2, depending upon the nature of the substance which is being utilized. The respiratory quotient for glucose is 1.0 which means that the volume of CO_2 produced equals that of the O_2 consumed. It follows therefore that bacteria and protozoa are not inhibited by such concentrations of CO_2.

The effect of increased concentrations of CO_2 is more marked with protozoa than with bacteria. Cultures of Trypanosoma lewisi failed to grow in 22 per cent CO_2. Leishmania tropica grew well in 20 per cent CO_2 but failed to develop in 29 per cent. The trypanosome culture is more sensitive than Leishmania to increased concentration of O_2 or of CO_2.

In testing the action of high concentrations of CO_2, it is very desirable that the containers shall each receive about 100 c.c. of O_2 per tube of culture to insure abundant O_2 for development. This necessitates the use of larger jars in tests with high CO_2 concentrations than are needed for those with lower tensions. When sufficient oxygen is provided the tubercle bacillus will grow even in 90 per cent CO_2. The growth, however, is slow to develop and is not as rich as in 90 per cent O_2. In general, it may be said that concentrations of CO_2 up to 50 per cent have little or no effect upon the growth of the tubercle and glanders bacilli. At 60 per cent and above the respiratory rate is decreased and as a result the growths are less rich. No difference, however, is observed in the respiratory quotients.

The effect of low concentrations of CO_2 is of no particular significance for

it is conceivable than an organism may grow in the open air in which case the CO_2 produced would be dissipated about as fast as made and of course the tension of CO_2 in the air (0.04 per cent) would be unaffected. Indeed it is possible to obtain an excellent growth in an atmosphere in which the tension of CO_2 is apparently nil. This fact was brought out in the following test: a glycerine agar plate, inoculated with the bovine tubercle bacillus, was placed in an inverted position, without the cover, in a jar through which CO_2-free air was forced at the rate of 600 c.c. per minute for twenty-one days. No CO_2 could be detected by buret analysis and yet a very rich culture developed. It would be wrong to conclude from a test such as this that the organism grew in a CO_2-free atmosphere. As a matter of fact the germ grew in a film of moisture which was not CO_2-free. The condition parallels growth in a broth culture over alkali (Table XIII).

Complete Removal of CO_2.—A considerably more important question is the growth of organisms in the absence of CO_2. Wherry and Ervin were the first to show that the tubercle bacillus failed to grow in the presence of alkali. In other words the removal of CO_2 from a culture resulted in inhibition of growth. This observation was confirmed and extended by Rockwell and by Valley and Rettger. The interpretation of their results, however, was open to question, since it seemed that something more was involved than the mere removal of CO_2 from the air and medium. It was conceivable that intracellular CO_2 was an essential in cellular equilibrium, and hence, in the respiratory process. Its complete withdrawal from a cell would therefore alter the equilibrium and as a consequence respiration would cease and death result.

Definite evidence of the presence and amount of intracellular CO_2 in bacteria has been wanting. Accordingly, tests were made with human and bovine tubercle bacilli, with a saprophytic tubercle bacillus and with the hay bacillus. The results obtained with the latter organism are presented in Table XII where it will be seen that in 1.9 gm. of the thoroughly dried cells only 0.2 or 0.32 c.c. of CO_2 was present. The same amount of cells, in the moist washed condition, held 1.72 c.c. of intracellular CO_2 the major part of which is lost by desiccation over alkali. Small as is this amount of CO_2 it nevertheless appears to be of great significance in the viability of the cell.

TABLE XII

TOTAL INTRACELLULAR CO_2 IN WET AND DRY B. SUBTILIS. C.C. AT 0° C., 760 MM.*

EXPERIMENT NUMBER	3	4
CO_2 in air control	. . . .	0.09
CO_2 in washed cells	1.44 (5)	1.72 (5.3)
CO_2 in dried cells	0.22 (5)	0.20 (5.3)
Weight of dried cells	. . . .	1.91 g. (5.3)
CO_2 present	. . . .	0.32

*The figures in parenthesis indicate number of Roux flasks used.
Cells dried in N_2 over P_2O_5 and KOH for two and thirteen days respectively.

While under certain conditions growth can be inhibited on the surface of a solid medium, such as agar or serum, it is not possible to bring about this result with an inoculated broth. This is shown in an experiment, the results of which are given in Table XIII. In this test the inoculated broth, in an open

TABLE XIII

CULTURE OF B. SUBTILIS IN GLYCEROL BROTH OVER ALKALI AND OVER WATER*

	KEPT OVER		
	ALKALI		WATER
TEST NO.	1	2	3
Inoculated broth			
Gaseous CO_2	0	0	1.12
Dissolved CO_2	1.04	1.32	2.56
Control			
Uninoculated broth			
Gaseous CO_2	0		0.04
Dissolved CO_2	0.60		0.88

*The figures represent volume per cent of CO_2.

Petri dish, was set in a jar. Alkali was placed above and below the culture dish to remove the free gaseous CO_2. Analysis showed the absence of CO_2 from the air of the jar and yet a typical rich growth developed in the broth. Analysis of the broth, however, revealed the presence of 1.04 and 1.32 volume per cent of CO_2. Th alkali was able to effect complete removal of CO_2 from the air in the jar but was not able to do this to the broth itself. The organism consequently was exposed to a certain tension of dissolved CO_2 although the overlying air was practically free of the gaseous form. It is evident from this and like tests that, in the presence of alkali or streaming CO_2-free air, even a film of water will favor multiplication, presumably by preventing a rapid loss of intracellular CO_2.

There are other factors besides a relatively dry surface of the solid medium which must be taken into account in experiments designed to test the question of the growth of organisms in the absence of CO_2. The nutrient quality of the medium is likewise a determining factor. On the ordinary beef infusion agar it is quite impossible to secure inhibition of rapidly growing organisms by exposure to alkali. A growth once started will be wholly unaffected by the presence of an absorbent. The less nutritive extract agar by prolonging somewhat the lag period favors the result sought. Adjustment of the reaction to the acid side, such as P_H 6.0, is of distinct value. The typhoid bacillus, in the presence of alkali, grows unhindered on infusion agar but is completely inhibited on extract agar P_H 6.0.

The use of a broth suspension of the organisms to be tested is undesirable, because it furnishes a readily available nutrient medium. Moreover, even if quickly dried, it forms a protective film around the organisms. Hence, it is preferable to use as the inoculum suspensions made with distilled water. A drop or two of this water suspension is placed on the dry surface of the medium and spread about by a bent glass rod so as to secure as far as possible perfect distri-

bution. This cannot always be accomplished and some piling up of cells in minute masses may occur. Such grouping of cells affords mutual protection and as a result colonies may develop on the medium in the presence of alkali.

By observing the conditions mentioned it is possible to obtain complete inhibition of growth when the inoculated medium is exposed to the action of an absorbent. An alkali, however, is not necessary for the same result can be secured by exposure to a large volume of N_2, as in an autoclave; or to streaming nitrogen; or to streaming CO_2-free air; or in a vacuum.

Inhibition of growth, however, is not the most significant fact arrived at by these tests. If, after a given exposure, the plate is taken out of the apparatus and set aside in the incubator, it will be found that rapid destruction of the organism has taken place.

This reduction in viability will be noted in Table XIV. In these experiments the hay bacillus was exposed, over alkali, in nitrogen. No visible growth developed. But when removed and incubated in the air, after an exposure of four hours, the plate showed an appreciable decrease in the number of colonies as compared with the control plate. The reduction was very marked at the end of eight hours and after 12 hours was nearly complete.

TABLE XIV

REDUCTION IN VIABILITY OF B. SUBTILIS BY EXPOSURE OF INOCULATED PLATES IN N_2 OVER ALKALI AT 33° C.*

OVER ALKALI IN N_2	THEN CHANGED TO OVER WATER IN AIR PLATES GAVE
Hours	
4 (2)	100 colonies each
8 (2)	4 and 7 colonies
12 (3)	0, 2 and 4 colonies
24 (3)	0 (2) and 3 colonies
36 (7)	0 (6) and 2 colonies

*The figures in parenthesis indicate the number of tests.
Control plates developed 800 or more colonies.

The same result was obtained with plates exposed to streaming nitrogen or to a vacuum without the presence of alkali, as shown in Table XV. The same is true when streaming CO_2-free air is used.

TABLE XV

REDUCTION IN VIABILITY OF B. SUBTILIS BY EXPOSURE OF INOCULATED PLATES IN NITROGEN OR IN VACUUM AT 33° C.*

	HOURS	THEN CHANGED TO AIR OVER WATER GAVE
In streaming nitrogen	8 (2)	150 colonies
	12 (2)	100 colonies
	24 (2)	0, 3 colonies
In continuous vacuum	8 (2)	100-200 colonies
	12 (2)	50- 70 colonies
	24 (6)	0- 18 colonies

*The figures in parenthesis indicate number of tests.
Control plates developed 800 or more colonies.

In considering the effect of moisture, an experiment was cited in which CO_2-free air, streaming at the rate of 600 c.c. per minute, failed to inhibit the growth of the tubercle bacillus. The same was true for other organisms tested in like manner. It became evident that the effect, if any, of a stream of CO_2-free air could only be obtained by passing it directly over the inoculated surface. The Petri plates were therefore discarded and instead Kitasato flasks were used. This modification gave at once the expected results.

TABLE XVI

INHIBITION AND DESTRUCTION OF B. SUBTILIS IN STREAMING CO_2-FREE AIR, KITASATO FLASKS, EXTRACT AGAR, P_H 6.0, 37° C. AEROBIC GROWTH IN SLOW STREAM. PROTECTIVE ACTION OF CO_2.*

RATE OF FLOW C.C. PER MIN.	EXPOSURE HOURS	CO_2-FREE AIR		CO_2-FREE AIR +3% CO_2 GROWTH
		PRIMARY COLONIES	THEN PLACED IN AIR 35° C. 48 HRS.	
200	12	0	7	+ rich
200	24	0	0	+ rich
100	24	1	2	
50	24	0	0	
25	48	0	0	
10	48	0	0	
4	48	0	0	
1	24	+ like control	+ like control	

*The control plates had 200 or more colonies.
The colonies which developed during the exposure period are designated as "primary."
The total number of colonies present on the plate, after incubation in ordinary air, indicates the number of survivals.

Table XVI which shows the effect on the hay bacillus is illustrative of the results obtained with a number of other organisms. With the CO_2-free air streaming at the rate of 200 c.c. per minute, there was complete inhibition, and the flask which was thus treated for twelve hours on subsequent incubation gave only 7 colonies, while that which was exposed for twenty-four hours gave none. Practically, all of the organisms were therefore killed in less than twelve hours. The presence of only 7 colonies in one case is probably due to the almost unavoidable grouping of cells in the process of spreading. A massing of this kind was responsible, without doubt, for the presence of 1 primary colony in the flask aerated at the rate of 100 c.c. per minute.

By dividing the current of CO_2-free air, it is possible to run duplicate tests or, what is better, to introduce into one branch a slow stream of CO_2. As shown in Table XVI, the restitution of CO_2 to the main stream of CO_2-free air resulted in a rich growth the same as in a control flask kept in the room. The rate of flow was the same through both flasks. In the one which received CO_2-free air, there was no growth and death resulted. In the other flask through which passed the same air plus 3 per cent CO_2, there was a rich growth.

A striking demonstration of the effect of absence or presence of CO_2 was obtained with the human and bovine tubercle bacilli which were tested side by side. A ground up suspension in distilled water of these organisms was spread over the previously dried surface of infusion agar P_H 7.4 in Kitasato flasks. The rate of flow was 100 c.c. per minute. The flasks through which streamed CO_2-free air, plus the addition of 6 per cent CO_2, at the end of twenty-eight days showed solid growths even richer than an ordinary control. On the other hand, the flasks through which only CO_2-free air passed showed no evidence of growth at the end of twenty-eight days. They were then disconnected and set aside in the incubator at 37° C. for one hundred twelve days but no sign of growth appeared. Total destruction of the two strains of tubercle bacilli was brought about by the streaming CO_2-free air.

The high speed of aeration alone was not responsible for the destructive effect. As shown in Table XVI the reduction of the speed to 4 c.c. per minute gave the same result, viz., destruction of the organism. With a speed of 2 or 3 c.c. per minute many primary colonies developed and on subsequent incubation the growth became the same as on the control plate, showing that no destruction took place. With a speed of 1 c.c. per minute the flask developed a growth like that of the control. Incidentally it may be stated that in a similar slow stream of purified CO_2-free nitrogen the hay and the typhoid bacillus grew as facultative anaerobes.

It is evident therefore that growth can and does take place in a slow stream of CO_2-free air. This result is to be expected if it is supposed that the observed injurious effects are due to the depletion of intracellular CO_2. In a rapid stream the organism is losing its reserve of this gas faster than it is being made, whereas in a sufficiently slow stream whatever loss occurs by diffusion through the cell wall is overcompensated by the respiratory process. Hence the normal intracellular equilibrium is maintained and growth results.

It has been held that CO_2 was utilized as a food and that the failure of organisms to grow on plates exposed to alkali was due to the removal of CO_2 from the medium. This view, however, is not supported by experimental facts. Thus, if an uninoculated agar plate is kept over alkali for twenty-four to forty-eight hours it should be rendered free of the supposedly nutrient CO_2 and consequently would become a poor medium for growth. Again, if through an uninoculated Kitasato flask a stream of CO_2-free air (100 c.c. per minute) is passed for two to three days it likewise should be made unfavorable as a culture medium. But in either case, after the exposure mentioned, if the medium is quickly inoculated and at once subjected to a slow stream of CO_2-free air (1 to 2 c.c. per minute) growth results the same as on a control.

Clearly, it is not the medium which is affected by exposure to an absorbent or to a rapid stream of CO_2-free air. The reasonable conclusion to be drawn is that these agents deprive the inoculated organism of an essential constituent, viz., intracellular CO_2.

It is well known that some organisms, such as the bovine strain of B. abortus, do not readily grow in the primary isolation unless some CO_2 is introduced into the culture jar. The explanation of this peculiar behavior, in the light of

these studies, is that the organism on removal from the body loses a considerable amount of its intracellular CO_2 and its growth is thereby retarded. This loss is compensated by placing the inoculated tubes under increased CO_2 tension, and as a result good growth takes place.

The fact that a rapid stream of CO_2-free air or nitrogen, or exposure to an absorbent, destroys organisms can perhaps be utilized to distinguish between the living cell and nonliving inanimate matter. For example, the question of the nature of the bacteriophage has been much discussed. If it be a living organism it should be killed by exposure to conditions which effect removal of intracellular CO_2. A nonliving chemical substance, an enzyme, for example, might be expected to withstand such conditions. In the tests which have been made thus far, it has not been possible to decrease or destroy the bacteriophage by CO_2-free air. This fact speaks against the view that the lytic principle is a living organism. Further work, however, is necessary before a final conclusion can be drawn.

SUMMARY

By means of exact methods it has been possible to determine the extent of gas exchange for a number of bacteria and protozoa. Respiration is an essential characteristic of living matter, and in the case of aerobic organisms it is evidenced by the consumption of oxygen and the elimination of CO_2. The rate and intensity of this change varies with the composition of the medium and the kind of organism. The respiratory quotients in general approach the theoretical values. The effect of varying concentrations of oxygen and of carbon dioxide are shown. The reduction or removal of intracellular carbon dioxide results in the death of the cell and this fact perhaps may be used in determining the nature of the so-called filtrable viruses.

A COMPARATIVE STATISTICAL STUDY OF THE FREQUENCY OF ASYMPTOMATIC RINGWORM AS OCCURRING IN THE MORE COMMON CUTANEOUS AFFECTIONS*

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THIS investigation has a threefold purpose: an evaluation of the disputed microscopic forms of ringworm hypomycetes; demonstration of some possible relationship between asymptomatic ringworm infection and certain of the common dermatoses; and lending further support to the potential possibilities of the silent (asymptomatic) form of mycotic infections of the feet as a causative factor in the production of cutaneous disease. The lack of appreciation of the importance of this problem probably rests upon the skepticism concerning the pathogenicity of some of the forms of ringworm fungi found in the silent type: doubt as to the true nature of the "mosaic" type of ringworm hypomycetes; belittlement of the comparatively insignificant lesions of asymptomatic epidermophytosis; and failure of realization that the lesions of the silent type may assume active (symptomatic) form.

Both the clinician and the laboratory worker have attempted to define definitely the status of this problem. The clinician, with full realization of the frequency with which fungi (the ringworm hypomycetes) are etiologically responsible for vesicular and squamous eruptions of the hands and feet, has not failed to take into consideration the causative possibilities of the various occupations ("Occupational dyshidrosiform dermatitis of Darier"), as well as the influence of disfunctions of the sympathetic nervous system in the production of eruptions closely simulating those caused by the ringworm hypomycetes. The laboratory has shown that yeasts and even cocci may bear an etiologic relationship to cutaneous lesions similar to those produced by ringworm fungi.

As the "mosaic" spores are frequently encountered in microscopic preparations of epidermal tissues of asymptomatic epidermophytosis, their exact status becomes a matter of greatest importance. Becker and Ritchie believe that the "mosaic" type represents a collection of organic material which may result from inflammatory changes in the tissues. These authors were able to produce forms simulating the "mosaic" configurations through admixture of potassium hydroxide and olive oil, but the double contour was lacking. F. Weidman concludes that he does not believe all of the mosaic forms to be true forms of fungus. Greenwood and Rockwood, however, are convinced of "the identity of the somewhat bizarre, degenerate 'mosaic' form with the usual actively growing mycelium." They were able to demonstrate the continuity of these forms with normal mycelium in the same scale.

G. M. MacKee and G. M. Lewis in a recent contribution entitled, "Keratolysis Exfoliativa and the Mosaic Fungus," consider the question of the

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"mosaic" fungi at great length and the following are the most important of their conclusions:

1. The "mosaic" fungus is frequently demonstrated in various types of dermatomycoses in association with accepted forms of pathogenic fungi.

2. "Mosaic" fungi are rarely demonstrated in diseases other than the conventional dermatomycoses or dermatophytid of the hands and feet.

3. Pathogenic growths have been cultivated in a few instances from the scales and vesicular tops of ringworm lesions when only "mosaic" forms could be demonstrated in extemporaneous preparations.

4. Artificially produced blisters gave negative results for fungus, excepting in locations usually predisposed to dermatomycoses with the patients giving

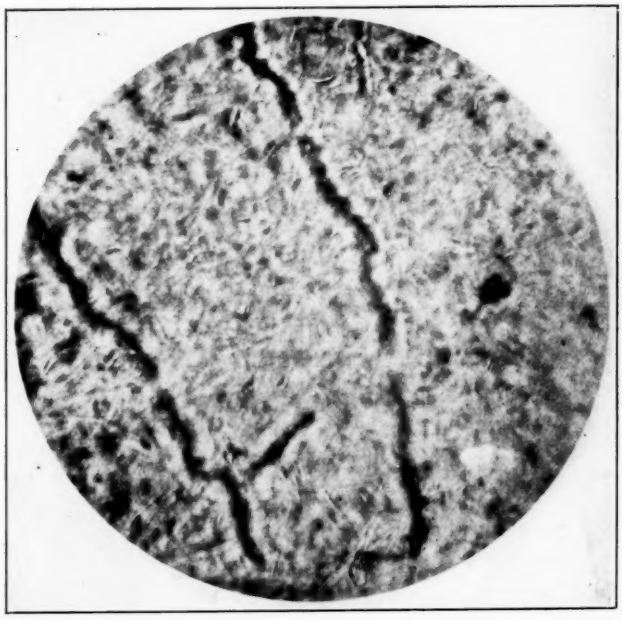

Fig. 1.—Mycelia found in epidermal tissue of vesicle before treatment January 21, 1931.

either the history of a previous attack of dermatomycosis or of a fungus focus located either on the hands, feet, or between the toes.

5. Use of zylene preparations and the absence of the "mosaic" forms in the fatty scales of seborrhea seem to disprove that these forms are artifacts, the result of chemical union of fat globules with potash solutions.

6. If a case of dermatomycosis is examined repeatedly for a long while, the conventional hyphae may be found at one time, the "mosaic" form at another, or both may be found in the same specimen.

In this connection the following clinical and laboratory observations of one of us (A. S.) are worthy of record.

M. G. consulted (A. S.) for a skin condition involving the palms and fingers of both hands; duration nine months. The skin was thickened, fissured, scaly, and presented a picture considered typical of eczema squamosum. Repeated examination of the scales removed from the hand lesions proved negative for fungi.

The feet showed but little pathology, excepting for slight scaling of the plantar surface of the left big toe. Repeated examination of the epidermal scrapings of the feet lesions showed only "mosaic" spores. After three weeks' treatment the affected skin surface of the feet appeared clinically normal, and microscopic examination of the removed material showed absence of fungus. The active lesions of the hands showed marked improvement under treatment. About five weeks after beginning treatment, the condition of the hands seemed to remain stationary and almost coincidental with this, the patient complained of an eruption on the plantar aspect of the left big toe. Examination disclosed a few vesicles in this area together with rather marked scaling. Microscopic study of the vesicular type showed the presence of typical mycelia.

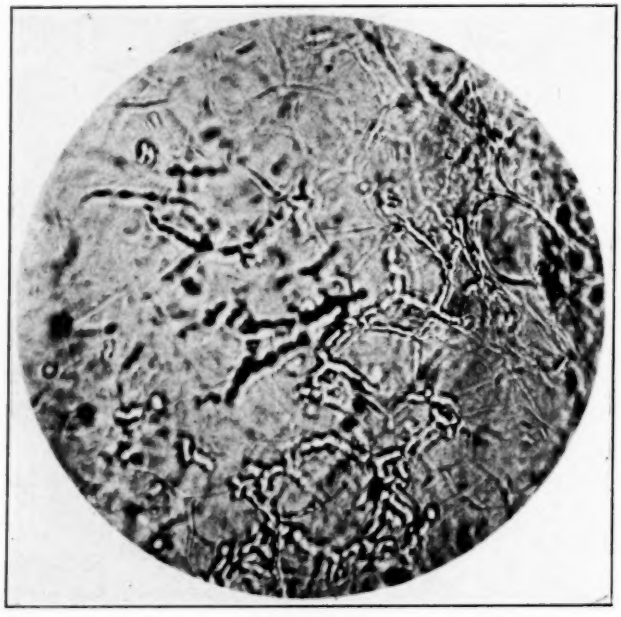

Fig. 2.—"Mosaic" spores found in epidermal tissue of same vesicle after treatment January 24, 1931.

A. W. presented as part of his cutaneous eruption a few typical steel-colored vesicles on the inner aspect of the right big toe. Opportunity was offered (A. S.) to observe these lesions daily and to remove material for study. The following photographs show the mycelia of the active stage as well as the "mosaic" spores of the rather unfavorable environment created through treatment.

The evidence cited here, together with the observations of Greenwood and Rockwood, and MacKee and Lewis form to our minds uncontradictory evidence that "mosaic" spores are a true form of ringworm hypomycetes.

In addition to the consideration of the status of the "mosaic" spores one of the objectives of this investigation was the determination of the pathogenicity of the fungi of the silent form of feet mycosis. Only one of Koch's postulates was studied, i.e., ability to grow the causative organism in artificial culture media. Epidermal scrapings from each of the 185 patients studied were cultured in

Sabouraud's media according to the usual technic. Dr. Ralph P. Zalatel, Pathologist of The Skin and Cancer Hospital of Philadelphia was responsible for the cultural findings.

Analysis of the data obtained in this investigation justify the following statements.

One hundred and eighty-five patients were examined, and out of this number, 92, or a trifle over 49 per cent were positive for fungi on extemporaneous examination, while 93, or a portion over 50 per cent were negative. Only in 4 instances, or in a little over 2 per cent was it possible to obtain a typical ringworm growth on Sabouraud's media.

The last phase of this investigation, an attempt to associate asymptomatic epidermophytosis with one or another of the more common dermatoses is here presented with the following results:

Forty-five patients with acne vulgaris were examined for ringworm fungus infection of the feet. Their ages ranged from thirteen to twenty-nine years. Of this number, 27 or 60 per cent showed negative microscopic findings for fungi, while 18, or 40 per cent proved positive. Epidermal material from the interspaces of all of the 45 patients were cultured on Sabouraud's media and in only one instance was a typical ringworm fungus growth obtained. Scaliness of the interspaces was present in all cases, except in one instance where the interspaces appeared to be apparently normal. Exfoliation was an additional feature in 28 out of 45 patients studied. In the 27 negative fungus cases, 14 showed involvement of one to three interspaces and 13 cases showed involvement of all the interspaces. Of the 18 positive cases, 9 showed involvement of one to three interspaces and 9 showed involvement of all interspaces.

Fourteen patients with eczema were studied; 5 of the vesicular variety, 6 of the papulo-squamous type and in 3 instances the type was not given. Of this number, 3 or 22 per cent were found positive on microscopic fungus examination and 11 or 78 per cent were negative. All failed to give a typical ringworm growth on Sabouraud's media. Out of the 14 patients, 7 showed involvement of all the interspaces and 7, one to three interspaces. Scaliness was present in all of the patients with eczema. Exfoliation was found in only 7 instances.

Ten patients with seborrheic dermatitis were studied as follows:

Five or 50 per cent were positive for fungi on extemporaneous examination and 5 or 50 per cent negative. Specimens of all the 10 cases cultured on Sabouraud's media failed to give a positive result. In 6 instances all the interspaces were involved and in 4, only several. All cases showed scaliness of the interspaces.

The miscellaneous group included the following: 29 patients with dermatitis; 5 with seborrhea; 5 with psoriasis; 6 with herpes; 4 with urticaria; 4 with rosacea; 4 with scabies; 10 with various cutaneous pus affections; 5 with varicose ulcers; 4 with pruritus; 4 with alopecia; 3 with tertiary syphilis; 6 with various types of dystrophy of the skin; 3 with miliaria; 3 with lichen planus; and 15 with benign and malignant skin tumors.

This series constituted 110 patients and of this number 51 or 46.4 per cent were microscopically negative for ringworm fungi and 57 or 51.8 per cent posi-

tive. Only two of this group (1.8 per cent) gave positive cultures on Sabouraud's media.

Individual statistics of the more prominent cutaneous affections included in the above are shown in Table I.

TABLE I

NAME	NO. OF PATIENTS	PER CENT OF POSITIVE MICROSCOPIC FUNGUS FINDINGS	PER CENT OF NEGATIVE MICROSCOPIC FUNGUS FINDINGS
*Dermatitis	29	55.2	44.8
Seborrhea	5	40.	60.
Psoriasis and Lichen Planus	8	12.5	87.5
Scabies	4	50.	50.
Varicose Ulcers	5	20.	80.
Tumors	15	53.33⅓	46.66⅔
Pus affections	10	60.	40.

*In one case of dermatitis a positive culture on Sabouraud's media was obtained.

RÉSUMÉ

1. One hundred and eighty-five patients suffering from various skin affections were examined for the presence of ringworm fungi in the interspaces of the feet, both by the extemporaneous method and by culture on Sabouraud's media.

2. Of this number, 92 or 49 per cent were proved positive for fungi on microscopic examination and 93 or 51 per cent negative.

3. Only in 4 instances or 2 per cent could a positive ringworm growth be obtained on Sabouraud's media.

4. Forty-five patients with acne vulgaris were studied. Of this number, 27 or 60 per cent were negative for fungi by the extemporaneous method, while 18 or 40 per cent were positive. Out of the 45, there was only one case which gave a positive ringworm growth on Sabouraud's media.

5. Fourteen patients with eczema were studied with the following results: Three or 22 per cent were positive for fungi by the microscopic method and 11 or 78 per cent were negative. All failed to give a ringworm growth on Sabouraud's media.

Eight patients with either psoriasis or lichen planus were studied, and of this number 12.5 per cent showed positive microscopic findings and 87.5 negative results.

6. Ten patients with seborrheic dermatitis were examined. Of this number 5 or 50 per cent were positive for fungi by microscopic examination and 5 or 50 per cent negative. All failed to yield positive cultures on Sabouraud's media.

7. Patients with various forms of dermatitis, scabies or tumors showed about an equal proportion of positive and negative microscopic findings.

8. While the general average of positive fungus findings by microscopic examination was 49 per cent for the entire series, the eczema group only yielded 20 per cent positive results, acne vulgaris 40 per cent, but the seborrheic dermatitis group gave 50 per cent positive. Although this series is rather small upon which to base definite conclusions, it would appear that the eczema patients seem

to offer the least favorable soil for asymptomatic epidermophytosis and seborrheic dermatitis patients the most favorable environment.

9. Statistical study of the frequency of asymptomatic mycosis of the feet is well recognized. However, doubt as to the pathogenicity of the ringworm fungi encountered in this affection still remains. This investigation is an attempt to show that these fungi, through their ability to grow in favorable culture media outside the body probably possess potential powers of assuming the same rôle in the human skin, when conditions become favorable. Since it was found impractical to fulfill all of Koch's postulates, particularly reinfection of human beings with ringworm fungi, this work was not carried on beyond the attempt to culture on Sabouraud's media.

10. An important lesson to be drawn from this paper is the need for a test, cutaneous or otherwise, through which the potential, latent pathogenic powers of the fungi of asymptomatic ringworm fungus infection could be determined. By such a method it would also be possible to reconcile the discrepancies between the cultural and microscopic findings of asymptomatic mycosis.

CARBOHYDRATE IN THE TREATMENT OF POSTOPERATIVE TETANY, WITH SPECIAL REFERENCE TO LACTOSE*

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THE usual methods of treatment of postoperative tetany are not entirely satisfactory. To any who have experienced the difficulties in the management of this condition, the need of simpler effective measures is apparent. The oral administration of calcium, even in large doses, is not always sufficient to control the symptoms, and repeated injections of it over long periods is undesirable or impossible. Injections of parathyroid extract alone or in addition to calcium may be effectual, but are inconvenient and expensive. Methods are reported here for the control of phosphate metabolism to an extent that will afford distinct benefit to patients suffering from this disease.

SERUM CALCIUM

The best-known and perhaps the most satisfactory single criterion by which the severity of parathyroid tetany may be judged is the degree of depression of the level of total serum calcium. Examination of a large series of serum calcium levels in tetany makes the fact apparent, however, that the symptoms do not necessarily parallel the total calcium levels. This confirms the opinion of John.[1]

It is known that the total serum calcium can be raised and frequently brought to normal in tetany by the feeding of large doses of calcium. In some of our cases the symptoms were not controlled by these measures, and their severity was thought to be out of proportion to the calcium level. This was especially striking since a patient might be symptom-free with a certain calcium level on one day, while on another occasion, although the serum calcium was at the same height, symptoms might be present. Abnormally high levels of blood phosphates accompanied nearly normal calcium values in some of these cases.

BLOOD INORGANIC PHOSPHATES

Ver Ecke[2] in 1898 noted a lessened phosphate excretion in the urine in tetany. This has received ample confirmation by Salvasen,[3] Greenwald,[4] and others, and it is now recognized that one of the constant features of parathyroid tetany is phosphate retention. Furthermore, tetany has been produced experimentally by feeding phosphates.[5] The phosphate retention in tetany usually is associated with a distinct rise of blood phosphates, the concentration of which partially governs the severity of the symptoms. This is definitely indicated by the observations reported here and by a review of the literature. Pronounced mitigation of symptoms accompanying a fall in blood phosphates has been ob-

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served, and simultaneously the neuromuscular electrical excitability approaches normal.

Calcium phosphate is a relatively insoluble compound. It has been stated that the blood is supersaturated with this salt.[6] This has been questioned,[7, 8] but it is certain that it approaches the saturation level. Under these conditions, it might be expected that a decrease in blood phosphates would result in an increase in serum calcium.[9] It has been observed more frequently in these studies, however, that there is a slight fall in total serum calcium accompanying the fall in blood phosphates. It is possible that the relief of symptoms associated with this fall in phosphates is the result of an increase in the percentage of calcium ionized. It is also possible that the decrease in phosphates independently decreases neuromuscular excitability.

RELATION OF CARBOHYDRATES TO BLOOD PHOSPHATES

At the beginning of this century it was generally accepted that phosphate metabolism was not normal in diabetes mellitus.[10, 11, 12] Since that time intensive study has demonstrated an extraordinarily close relationship between carbohydrate and phosphate metabolism. Harrop and Benedict[13] showed that in normal glucose-tolerance curves, the level of blood phosphate fell, the low point of the phosphate curve being subsequent to the highest glucose level. They believed that phosphates are utilized temporarily during the transference of glucose from the blood. This has been corroborated by many writers.[14, 15, 16]

It has been shown that in dogs suffering from tetany, the symptoms are more pronounced on a meat than on a carbohydrate diet. Dragstedt[17] states that dogs with tetany lived longer when fed milk, white bread, and lactose than the usual survival period for thyroparathyroidectomized dogs. In spite of insufficient evidence, his conclusions appear to be correct. Blood studies were not made in his series, and the improvement was considered to be the result of changes in the gut. That the Dragstedt diet is beneficial in tetany has received confirmation. Inouye[18] and Frank, Haring, and Kühnau[19] also contend that the beneficial effect of lactose is due to changes in the intestinal tract, since parenteral administration is not effective. This conclusion is not completely justifiable, for lactose is not absorbed as such, but is first hydrolized, with the formation of glucose and galactose. Hydrolysis takes place only to a small extent, however, if the lactose is given intravenously.[20]

Dragstedt believed that benefit was obtained in tetany by a diet of white bread, milk, and lactose because this diet prevented the absorption of toxic substances. Salvasen[3] criticizes Dragstedt's work, and in an excellent treatise presents evidence to prove that the entire benefit of milk is produced by its calcium content. He makes little comment on the effect of milk on blood phosphates. Although there is no question that calcium is essential, the studies herein reported make it apparent that the efficacy of this diet is partly attributable to its effect on phosphate metabolism.

In studying carbohydrate metabolism in parathyroidectomized dogs, Reed[21] found that not only ingestion but also injection of dextrose tends to alleviate symptoms of tetany, causing a decrease in inorganic phosphates and a less pronounced decrease in calcium.

THE OBJECT OF THE STUDY

From the preceding statements it is apparent that an increase in the level of inorganic phosphates in the blood may be closely associated with the production of symptoms in tetany. A fall in inorganic phosphates may cause alleviation of symptoms, even though it is not accompanied by a rise in serum calcium.

The object of this study was to find therapeutic measures which would lower the abnormally high level of blood phosphates, with the expectation that clinical improvement of the patient would result. Since there is such an intimate relationship between carbohydrate and phosphate metabolism, it seemed possible that the level of blood phosphates in tetany might be governed by the proper regulation of carbohydrate assimilation.

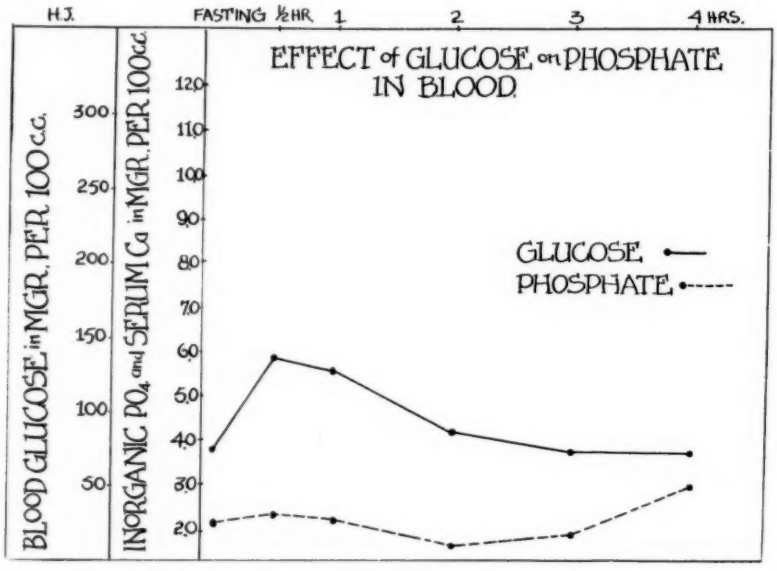

Chart 1

TABLE I

EFFECT OF GLUCOSE ON NORMAL INDIVIDUALS

TIME IN HOURS	FASTING	½	1	2	3	4
Case 224731						
Sugar, mg. per 100 c.c. whole blood.	83	155	154	119	52	75
Phosphate, mg. per 100 c.c. whole blood.	3.22	2.77	3.09	2.40	2.21	3.25
Case K. C.						
Sugar, mg. per 100 c.c. whole blood.	79	86	111	63	54	53
Phosphate. mg. per 100 c.c. whole blood.	3.64	3.07	3.31	2.58	2.31	2.78
Case L. H.						
Sugar, mg. per 100 c.c. whole blood.	99	113	115	88	90	90
Phosphate, mg. per 100 c.c. whole blood.	3.28	2.23	2.57	2.25	2.34	2.50

EFFECT OF GLUCOSE ON BLOOD PHOSPHATES IN NORMAL INDIVIDUALS

In Table I and Chart 1 are given results which confirm the finding that inorganic phosphate disappears from the blood after the administration of glucose in normal individuals.

The technic employed is as follows: A specimen of venous blood was obtained after the patient had been without food for twelve to fourteen hours. One hundred grams of glucose were fed, and samples of venous blood were obtained at intervals of half an hour and one, two, three, and four hours, respectively, after the administration of the glucose. Blood sugar and phosphate estimations were made on each sample of blood. Blood sugar was estimated by the method of Hagedorn and Jensen.[22] Phosphates were measured by the colorimetric procedure of Kuttner and Cohen[23] (Table I, Chart 1).

An examination of these results demonstrates that the lowest point on the phosphate curve usually appears later than the highest point of the sugar curve. Not infrequently the phosphates return to the normal fasting level before the end of four hours.

EFFECT OF GLUCOSE ON BLOOD PHOSPHATE, SERUM CALCIUM, AND NEUROMUSCULAR EXCITABILITY IN CHRONIC PARATHYROID TETANY

The method used was the same as that described above, with the following additions:

Serum calcium determinations according to the Clark and Collip[24] modification of the Kramer and Tisdall method were made on the fasting specimen and on two of the other specimens. The electrical neuromuscular excitability (Erb's sign) was measured immediately before obtaining each specimen of blood. The procedure was as follows:

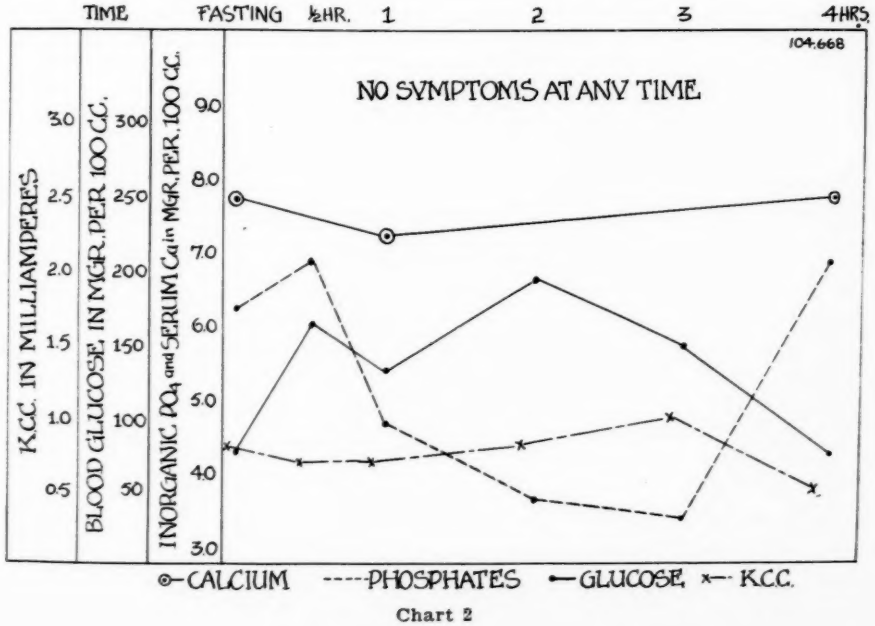

Chart 2

One of the patient's hands was placed on a large moist electrode connected to the anode of a circuit through which a variable direct current could be passed. The cathode consisted of a small metallic terminal covered with wet chamois. The skin over the median nerve at the wrist on the opposite side was moistened, and the electrode was applied. The circuit would be closed or opened by means of a small switch near the cathode. The amount of current running through the circuit was read from a milliammeter. In each case the current was gradually increased until a point was reached when, on closing the switch leading to the cathode, a contraction could be noted in the hand. The number of milliamperes necessary to produce the contraction was called the cathode closing contraction (K.C.C.). A decrease in this figure represents an increased irritability.

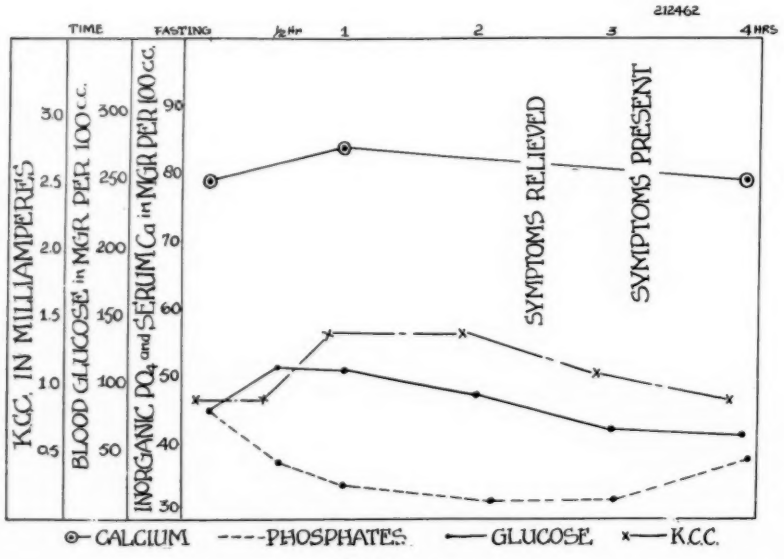

Chart 3

The patients on whom the following studies were made developed chronic parathyroid tetany following thyroidectomy. In each case Trousseau's, Erb's and Chvostek's signs were present, the latter only at times. Abnormally low blood calcium and high blood phosphate levels were always found, as shown in Table II. (Two cases which are not included in the tables are graphically depicted in Charts 2 and 3.) The blood sugar curves were essentially normal. The blood calcium changes were small and not constant. In four cases there was an increase, followed by a decrease. In two, there was a decrease which persisted until after the end of the experiment. In one case a decrease was followed by an increase, and in one there was no change. The phosphates invariably were decreased. Only in Case 3 did the decrease continue for as much as four hours. This was associated with a more prolonged rise in the blood sugar than in any of the other cases. The neuromuscular excitability showed an almost constant tendency to decrease during the test. Only in Case 5 was there a definite increase in excitability. In all cases in which there were symptoms at the beginning of the test the symptoms were definitely improved during the test, but in-

TABLE II

EFFECTS OF GLUCOSE ON CHRONIC TETANY

TIME IN HOURS	SUGAR MG. PER 100 C.C. WHOLE BLOOD	CALCIUM MG. PER 100 C.C. SERUM	PHOSPHATE MG. PER 100 C.C. WHOLE BLOOD	CATHODE CLOSING CONTRACTION	SYMPTOMS
Case 1, No. 222908					
Fasting	71	4.8	5.21	1.6	Present
½	133	—	4.74	1.6	Present
1	161	5.8	4.74	1.8	Improved
2	126	—	4.21	1.9	Improved
3	61	—	4.16	1.6	Present
4	59	4.8	4.87	1.4	Present
Case 2, No. 124350					
Fasting	65	6.8	6.50	0.8	Present
½	120	—	4.05	0.8	Present
1	87	6.8	5.30	0.7	Improved
2	87	—	3.60	0.8	Improved
3	58	—	3.00	0.7	Present
4	52	6.8	4.70	0.6	Present
Case 3, No. 222469					
Fasting	82	7.7	4.07	1.7	
½	142	—	3.30	1.9	
1	182	7.3	3.66	1.9	No definite
2	158	—	2.88	1.8	symptoms
3	117	—	2.42	1.8	
4	76	7.3	2.42	1.7	
Case 4, No. 220617					
Fasting	79	8.2	4.70	0.7	Present
½	154	—	4.70	1.2	Present
1	134	9.2	4.00	0.8	Improved
2	109	—	4.10	0.7	Improved
3	70	—	4.40	0.7	Present
4	54	8.2	4.10	0.7	Present
Case 5, No. 219959					
Fasting	83	7.8	5.50	1.6	Present
½	155	—	5.70	1.4	Symptoms
1	129	7.3	4.80	1.1	better
2	111	—	3.30	0.9	
3	68	—	3.45	1.1	Present
4	71	7.3	3.48	1.4	Present
Case 6, No. 109391					
Fasting	67	6.3	4.80	0.6	Present
½	81	—	5.60	0.7	
1	98	6.8	4.00	—	Gradual
2	89	—	3.20	0.6	Improve-
3	70	—	3.75	0.5	ment
4	70	6.3	3.75	0.4	Present
Case 7 (Control, no glucose given) No. 223213					
Fasting	73	7.3	6.82	0.9	
½	75	—	6.40	1.0	
1	79	7.3	7.50	1.0	
2	77	—	6.40	0.6	
3	72	—	6.82	0.7	
4	70	7.3	6.40	0.6	

creased in severity at the end of the experiment when the phosphate level rose. Apparently the symptoms paralleled the phosphate curve more than that of the serum calcium.

TABLE III

EFFECT OF LACTOSE AND GLUCOSE IN NORMAL DOGS

Dog No. 1, Weight 38, Experiment No. 1, Effect of Glucose, Dose 15 Grams

TIME IN HOURS	FASTING	½	1	2	3	4	5
Sugar mg. per 100 c.c. whole blood	92	101	132	95	92	84	—
Phosphate mg. per 100 c.c. whole blood	2.39	2.27	—	2.12	2.32	2.39	—
Calcium mg. per 100 c.c. whole blood	11	—	—	—	—	10	—

Dog No. 1, Weight 38, Experiment No. 2, Effect of Lactose, Dose 15 Grams

TIME IN HOURS	FASTING	½	1	2	3	4	5
Sugar mg. per 100 c.c. whole blood	77	74	117	75	66	75	—
Phosphate mg. per 100 c.c. whole blood	3.11	2.80	2.87	2.96	3.35	3.30	—
Calcium mg. per 100 c.c. whole blood	12.0	—	—	12.1	—	—	—

Dog No. 2, Weight 55, Experiment No. 1, Effect of Glucose, Dose 20 Grams

TIME IN HOURS	FASTING	½	1	2	3	4	5
Sugar mg. per 100 c.c. whole blood	77	90	119	77	74	72	—
Phosphate mg. per 100 c.c. whole blood	2.82	2.06	2.34	2.61	3.41	3.35	—
Calcium mg. per 100 c.c. whole blood	—	—	—	—	—	—	—

Dog No. 2, Weight 55, Experiment No. 2, Effect of Lactose, Dose 20 Grams

TIME IN HOURS	FASTING	½	1	2	3	4	5
Sugar mg. per 100 c.c. whole blood	79	79	79	75	81	81	79
Phosphate mg. per 100 c.c. whole blood	3.30	2.77	2.84	2.69	2.84	3.03	3.27
Calcium mg. per 100 c.c. whole blood	8.7	—	8.4	—	8.8	—	—

In Case 7 no glucose was administered. The results were included to show that the changes recorded in the other tables were caused by the glucose.

EFFECT OF LACTOSE ON BLOOD CALCIUM AND PHOSPHATE IN NORMAL DOGS

In one of the following sections it is shown that lactose is of great benefit in the treatment of tetany. The effect of feeding lactose to healthy dogs has been compared to the effect of feeding glucose to the same animals. The methods used were the same as those employed for glucose-tolerance tests. The results are shown in Table III. In this table, the blood sugar is expressed in terms of milligrams of glucose. The curve is very different after lactose feeding from that exhibited after glucose feeding. The glucose curves are similar to those in

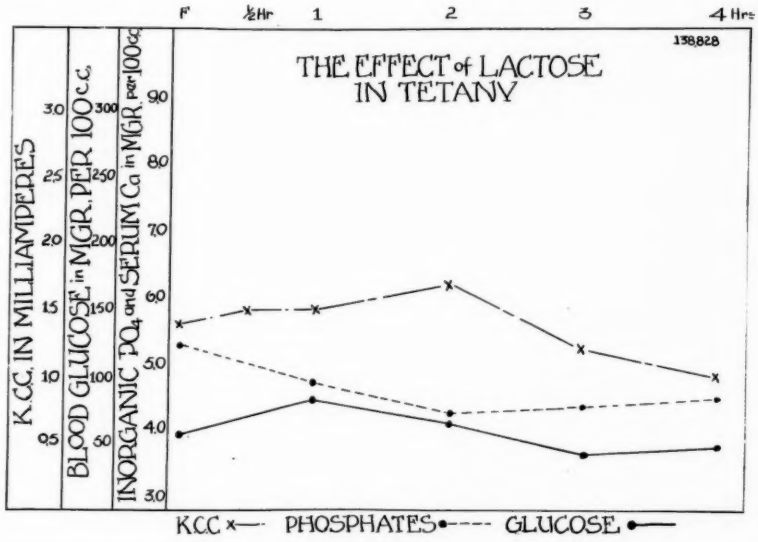

Chart 4

normal individuals; the lactose curves are very low, and indicate either a very high tolerance or very poor absorption. The serum calcium does not show a marked or regular change. There is no difference between the type of phosphate curve obtained with the two sugars, and no indication that lactose produces any prolonged depression of inorganic phosphate in the blood.

EFFECT OF LACTOSE IN CHRONIC TETANY

One hundred grams of lactose were administered to each of four patients suffering from chronic parathyroid tetany. The results are presented in Table IV and Charts 4 and 5. The blood glucose curves are of the same general type as those secured after the administration of glucose to normal individuals. The sugar values tend to be low. Again there is no definite effect on the serum calcium. The depression of inorganic phosphate is about the same as after glucose administration. It appears probable that the lactose is readily digested, with the formation of glucose and galactose, and that absorption takes place rapidly.

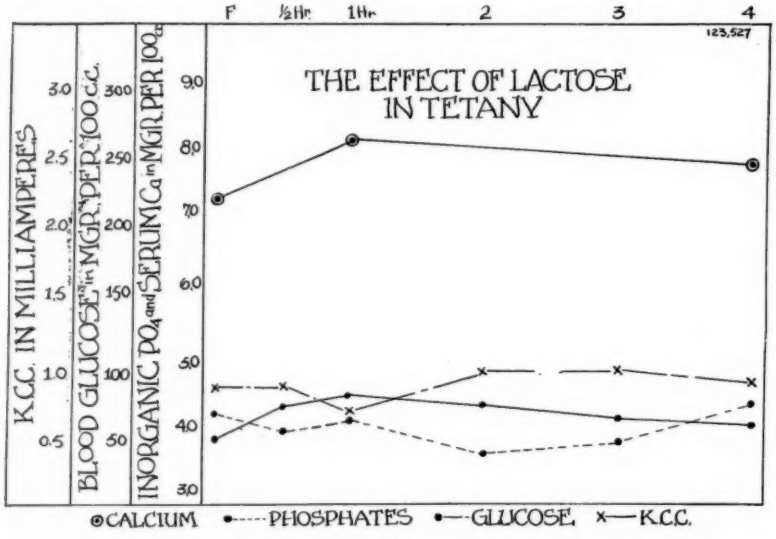

Chart 5

TABLE IV

EFFECT OF LACTOSE IN CHRONIC TETANY

TIME IN HOURS	SUGAR MG. PER 100 C.C. WHOLE BLOOD	CALCIUM MG. PER 100 C.C. SERUM	PHOSPHATE MG. PER 100 C.C. WHOLE BLOOD	CATHODE CLOSING CONTRACTION	SYMPTOMS
Case 8					
Fasting	77	7.7	5.00	0.7	Present
½	120	—	4.16	0.9	Present
1	117	7.2	4.16	0.7	Present
2	92	—	3.95	0.8	Improved
3	70	—	3.75	0.8	Improved
4	66	7.2	3.60	0.8	Improved
Case 9					
Fasting	65		3.85	1.0	Present
½	101		2.90	1.5	Present
1	77		3.56	1.4	Improved
2	77		3.56	1.8	Improved
3	48		3.82	1.1	Present
4	61		3.74	1.0	Present

EFFECT OF GALACTOSE ON BLOOD CALCIUM AND PHOSPHATE IN A NORMAL INDIVIDUAL

Since the glucose resulting from lactose digestion could account for all the changes in inorganic metabolism after lactose feeding, the effect of the galactose moiety was studied separately. The method was the same except that fifty grams of galactose were administered orally. It was considered advisable to use this smaller dose of galactose because of the low tolerance shown by most individuals to this sugar. In the following cases, sugar was excreted in the urine. The results are shown in Table V and Chart 6. As a control experiment, 100 grams of glucose were administered to the same individual (Chart 7). The effect of galactose on blood phosphate is in sharp contrast to that of glucose. This is in

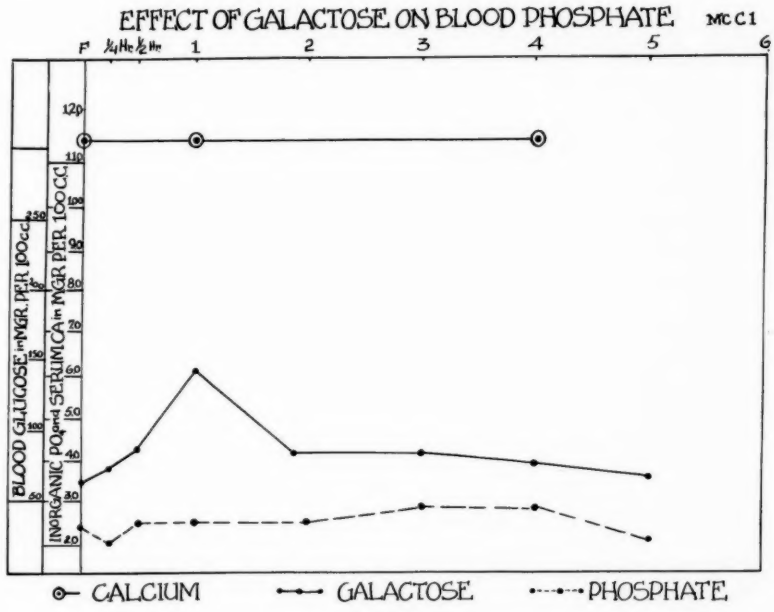
EFFECT OF GALACTOSE ON BLOOD PHOSPHATE
Mc C1
F ¼Hr ½Hr 1 2 3 4 5 6
BLOOD GLUCOSE in MGR. PER 100 c.c.
INORGANIC PO4 and SERUM CA in MGR PER 100 CC.
CALCIUM
GALACTOSE
PHOSPHATE

Chart 6

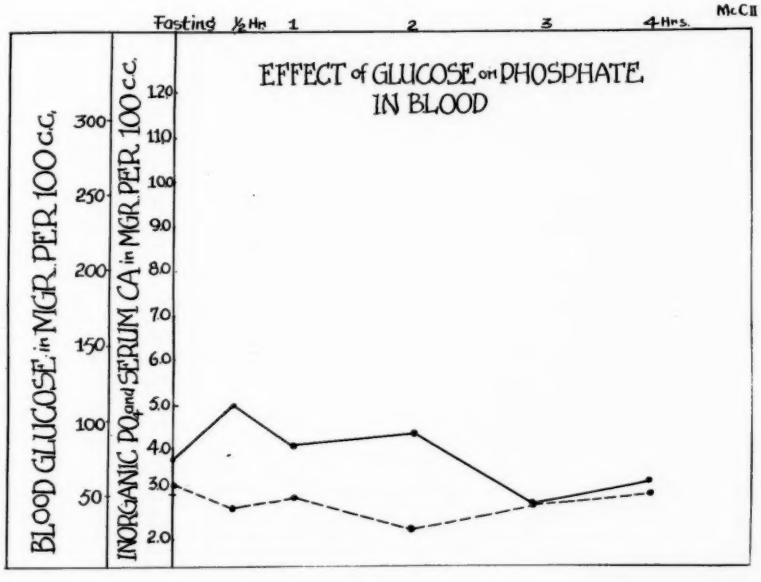
McC II
Fasting ½Hr 1 2 3 4 Hrs.
EFFECT of GLUCOSE on PHOSPHATE IN BLOOD
BLOOD GLUCOSE in MGR. PER 100 c.c.
INORGANIC PO4 and SERUM CA in MGR. PER 100 c.c.

Chart 7

accord with the results of Barrenscheen. Galactose does not produce a depression of the phosphate level in the blood.

EFFECT OF GALACTOSE IN CHRONIC TETANY

Galactose was administered also to two patients suffering from chronic parathyroid tetany, with the result summarized in Table V. Evidently there is no difference between the reaction to galatose of normal individuals and of those

TABLE V

EFFECT OF GALACTOSE IN CHRONIC TETANY

TIME IN HOURS	SUGAR MG. PER 100 C.C. WHOLE BLOOD	CALCIUM MG. PER 100 C.C. SERUM	PHOSPHATE MG. PER 100 C.C. WHOLE BLOOD	CATHODE CLOSING CONTRACTION	SYMPTOMS
Case No. 222908					
Fasting	83	7.2	3.76	1.5	No definite change in symptoms
½	95	—	3.66	1.8	
1	104	6.2	3.59	1.8	
2	97	—	3.68	1.7	
3	90	—	3.68	1.8	
4	83	7.6	3.81	1.8	
Case No. 167763					
Fasting	65	7.8	4.45	0.9	No definite change in symptoms
½	117	—	3.66	0.9	
1	95	7.9	3.55	1.0	
2	102	—	3.57	1.0	
3	83	—	3.64	0.9	
4	75	7.3	3.61	1.0	

with chronic tetany. No constant changes in inorganic metabolism appear following the administration of this sugar, the neuromuscular irritability does not show changes corresponding to those which occur after the administration of glucose, and the symptoms are not relieved during the test. Thus it seems improbable that it is the galactose moiety of the lactose molecule which results in depression of the blood phosphate. Further studies concerning the mechanism of the action of lactose on blood phosphate are in progress.

TABLE VI

EFFECT OF GLUCOSE, LACTOSE, AND GALACTOSE ON URINE PHOSPHATE EXCRETION

CASE NO.	DOSAGE	1 HOUR	2 HOURS	3 HOURS	4 HOURS
(a) In normal individuals					
L. H.	Glucose, 100 gm.	43.8	38.8	29.8	10.5
K. C.	Glucose, 100 gm.	65.9	66.2	58.3	39.6
172516	Glucose, 100 gm.	33.3	12.3	0.78	1.05
225005	Glucose, 100 gm.	32.1	1.16	1.65	0.95
McC.	Galactose, 50 gm.	33.2	12.9	27.3	21.0
(b) In chronic tetany					
138828	Lactose, 100 gm.	42.8	45.1	5.9	3.0
219941	Lactose, 100 gm.	21.3	5.2	20.2	1.5
177579	Lactose, 100 gm.	14.7	20.8	10.8	3.9
123527	Lactose, 100 gm.	22.1	17.1	4.9	13.8
222908	Galactose, 50 gm.	—	37.7	15.9	28.7
167763	Galactose, 50 gm.	5.2	5.7	4.0	5.4

EFFECT OF GLUCOSE, LACTOSE, AND GALACTOSE ON URINE PHOSPHATE EXCRETION

The fate of the inorganic phosphate which disappears from the blood stream has been considered, and it has been demonstrated that this phosphate is not excreted in the urine. In fact, when the blood phosphate is depressed after glucose or lactose administration, the phosphate excretion in the urine diminishes. After the administration of galactose, no definite change develops in the rate of phosphate excretion. This is to be expected, since galactose does not affect the phosphates in the blood stream. There is no difference between the reaction of normal individuals and of those with chronic tetany. The results are given in Table VI. Since the urine specimens were not taken with a catheter, the results given in this table are only approximations. The changes, however, are very marked and regular. It seems probable, from this work, that the phosphates which disappear from the blood are carried into the tissues.

LACTOSE IN THE TREATMENT OF CHRONIC PARATHYROID TETANY

In the following cases all calcium and phosphate estimations were made from samples of blood taken after the patient had fasted for approximately twelve hours.

CASE 1.—(109391.) A young woman eighteen years of age underwent thyroidectomy for adenoma of the thyroid in January, 1921. Two months after the operation she complained of paresthesia and stiffness of the fingers in attacks lasting from a few minutes to a few days. These symptoms persisted. She was treated by oral administration of calcium lactate at irregular intervals. On February 2, 1929, she complained of having had two severe tetanic convulsions. Examination showed the presence of Chvostek's and Trousseau's signs, and the serum calcium was 5.8 mg. per 100 c.c. From this date, 100 grains of calcium lactate were given daily. The symptoms were partially relieved, but mild symptoms continued.

Chart 8 shows that the average serum calcium level, taken at monthly intervals for a period of nine months previous to November 14, 1929, was 6.6 mg. per 100 c.c. On Novem-

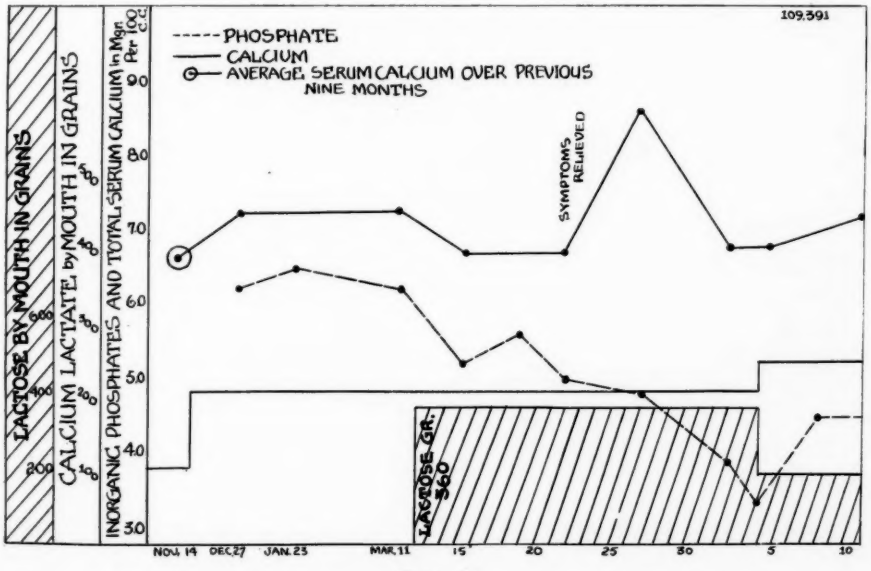
109391
PHOSPHATE
CALCIUM
AVERAGE SERUM CALCIUM OVER PREVIOUS NINE MONTHS
SYMPTOMS RELIEVED
LACTOSE BY MOUTH IN GRAINS
CALCIUM LACTATE BY MOUTH IN GRAINS
INORGANIC PHOSPHATES AND TOTAL SERUM CALCIUM IN Mgm. PER 100 C.C.
LACTOSE GR. 360
NOV. 14 DEC. 27 JAN. 23 MAR. 11
15 20 25 30 5 10

Chart 8

ber 14 the daily dose of calcium lactate was increased to 200 grains. The symptoms were improved slightly, and the serum calcium had risen to 7.2 mg. per 100 c.c. on December 27. On this date the inorganic phosphates were estimated for the first time, and were found to amount to 6.2 mg. per 100 c.c.

On January 23 the patient was placed on a high carbohydrate diet containing no meat, eggs, or cheese. In the hope of lowering the phosphate level, extra nourishment, consisting of candy, cake, biscuits, or fruit juices, was advised between meals and at bedtime. Between January 23 and March 11, she had five severe attacks of tetany, the symptoms always being more noticeable in the morning on wakening. Mild symptoms persisted between severe attacks, and the blood chemistry remained approximately the same.

On March 12, 1930, 360 grains of lactose per day were prescribed in addition to 200 grains of calcium lactate as before. These substances were divided into three doses, given before meals. The characteristic change in the chart consists in a persistent fall of the phosphate level. The symptoms were gradually alleviated and finally disappeared on March 27.

On April 4 the blood phosphates had fallen to a level which was lower than that considered necessary. The serum calcium was not normal, and therefore the dose of lactose was reduced to 180 grains and the calcium increased to 240 grains per day. The phosphate level rose, but remained within normal limits. The serum calcium also apparently rose somewhat. The temporary rise of serum calcium on March 27 is unexplained. The average serum calcium level was not greatly affected by the addition of lactose, and except for the one high value, there was a slight lowering of this figure until the calcium intake was increased on April 4. Since March 27 the patient has been completely symptom-free, with the exception of two days when she voluntarily discontinued treatment. On May 8 she stated that she was feeling better than she had for years.

CASE 2.—(219959.) A woman, thirty-four years of age, underwent thyroidectomy for adenomata of the thyroid with tracheal compression on November 21, 1929. The basal metabolic rate before operation was minus 16 per cent. On the morning following operation she complained of tingling in the fingers. Her serum calcium on this day was 8.6 mg. per 100 c.c.

At first the patient received calcium gluconate one dram three times a day, and calcium lactate, 20 grains three times a day. Her symptoms demanded the administration of

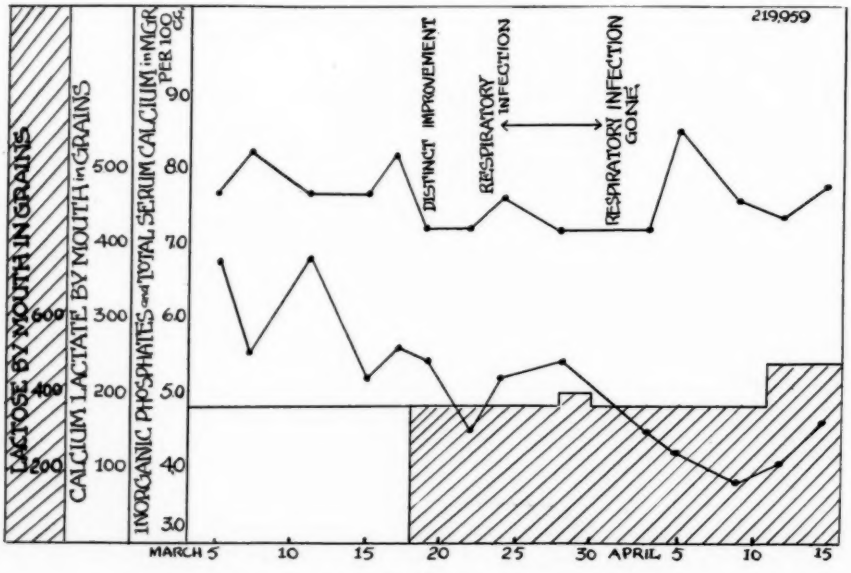

Chart 9

parathormone (parathyroid extract—Collip) on three occasions during the first thirteen days after operation, during which period the blood calcium fell to 7.7 mg. On December 4 the treatment was changed to one dram of calcium carbonate three times a day before meals. The symptoms were lessened in severity and the blood calcium rose from 7.7 mg. to 8.7 mg. on December 9, and to 9.2 mg. on December 12.

This treatment was continued, and the patient was not again observed until she returned on March 5, complaining that she had experienced almost daily stiffness and numbness of the fingers and twitching of the facial muscles. The serum calcium was then 7.7 mg. per 100 c.c. and blood phosphates 6.8 mg. as shown at the beginning of Chart 9. The treatment was changed on this date to calcium lactate, 180 grains per day, given in three doses of one dram each before meals. The serum calcium changed very little and the blood phosphate varied between 6.8 mg. and 5.2 mg. per 100 c.c. There were mild symptoms daily.

On March 18 the administration of lactose was started in doses of two drams three times a day before each meal. On the first day there was no improvement. On the second day the symptoms had disappeared, and on March 22 the blood phosphate was 4.5 mg. and the serum calcium 7.2 mg. On March 23 she developed a mild infection of the upper respiratory tract, associated with aching pains in the back and limbs. This lasted until March 30, and was accompanied by a slight rise in the blood phosphate level, but there was no paresthesia of fingers or toes and no stiffness of the fingers. Following March 23 the blood phosphate level continued to fall, and the patient felt entirely well.

It should be noted that while the patient's serum calcium varied slightly around the level of 8 mg. per 100 c.c., she had moderate symptoms of tetany associated with a high blood phosphate. Later, when the average serum calcium level was lower, the symptoms were greatly improved, associated with a fall in blood phosphates. It might be mentioned again that the blood phosphate levels given were estimated while the patient was fasting. Probably they were lower during the day than those shown in the chart.

Case 3.—(223213.) A woman aged thirty-two underwent a thyroidectomy for hyperthyroidism on February 20, 1930. Her basal metabolism before operation was plus 49 per cent. About thirty-six hours after the operation she complained of twitching of the facial muscles and paresthesia and stiffness of the fingers. Both Chvostek's and Trousseau's signs were present. The serum calcium on February 22 was 7.2 and on February 24 was 6.7 mg. per 100 c.c. The blood phosphate on this date was 6.2 mg. per 100 c.c.

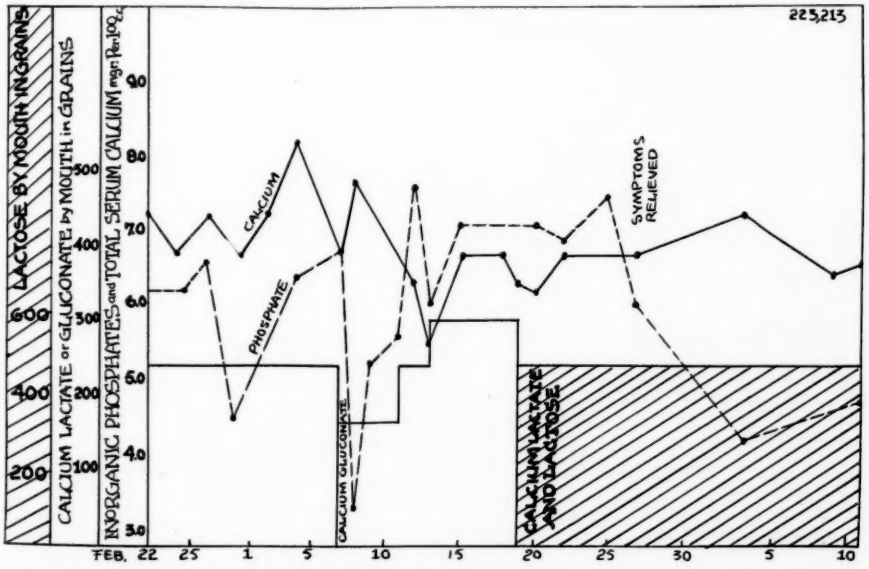
223,213
LACTOSE BY MOUTH IN GRAINS
CALCIUM LACTATE or GLUCONATE by MOUTH in GRAINS
INORGANIC PHOSPHATES and TOTAL SERUM CALCIUM mgr. Per 100 c.c.
CALCIUM
PHOSPHATE
CALCIUM GLUCONATE
CALCIUM LACTATE AND LACTOSE
SYMPTOMS RELIEVED
FEB. 22 25 1 5 10 15 20 25 30 5 10

Chart 10

Treatment was begun February 22 in the form of calcium lactate, 240 grains per day, 1 dram before meals and at bedtime, as shown in Chart 10. Symptoms were present daily, and the fasting serum calcium and blood phosphate varied as indicated.

On March 7, calcium lactate was discontinued and calcium gluconate was started, 180 grains per day in doses of 1 dram before each meal. The blood phosphate content fell markedly, but soon rose again to a higher level than before, in spite of the fact that the dose of calcium gluconate was raised first to 240 grains and later to 300 grains per day. The symptoms had disappeared on March 10, but on March 11 they reappeared and were more severe than before. Symptoms were present from the latter date until March 26.

On March 19 the treatment again was changed. The same amount of calcium lactate as was given previous to March 7 was prescribed. In addition to this, lactose was given in amounts of 480 grains per day, two drams being given with one dram of calcium lactate before each meal and at bedtime. For six days no distinct benefit was noted, but on March 27, the eighth day after the administration of lactose was started, there was definite improvement in the severity of the symptoms, accompanied by a fall in blood phosphates but without any rise in serum calcium. On this treatment the blood phosphates fell to a normal level, and symptoms disappeared entirely. The fasting blood phosphates are known to have remained normal for at least one month on this treatment, with the exception of two estimations done within one week following the extraction of an acutely abscessed tooth. On these occasions the phosphates were 6.4 and 6.0 mg., respectively. The patient was known to be symptom-free on May 8, 1930.

It is interesting to note again that the improvement in symptoms was associated with a fall in the level of blood phosphates but not with a distinct rise in the total serum calcium.

CASE 4.—(124350.) A woman thirty-four years of age underwent thyroidectomy for adenoma of the thyroid in June, 1923. The second day following this operation tetany developed. She experienced tingling, numbness, and stiffness of the fingers daily, and on occasions had severe generalized convulsions which were thought to be epileptic in character, but as the convulsions have not recurred since she has had adequate treatment for tetany, it is probable that they were caused by this condition.

From June, 1923, to the present time the patient has required constant treatment. At first, when she was taking 10 grains of calcium lactate and 1/10 grain of parathyroid extract twice a day, together with a mixture containing sodium bromide and tincture of hyoscyamus, she was not relieved, and continued to have symptoms daily and generalized convulsions occasionally. In May, 1925, when the calcium lactate was increased to 20 grains three times a day, she was somewhat relieved.

In June, 1926, injections of parathyroid extract (Collip) were begun, and a dose of 1 to 2 c.c. was administered subcutaneously every second or third day. In addition to this, she received parathyroid extract, grams 1/5, and calcium lactate, grains 10, three times daily, together with cod liver oil. In October, 1928, the intake of calcium lactate was raised to 120 grains per day. The patient felt better than she had since before the onset of the condition, but as moderately severe symptoms frequently were present, it was still necessary to give parathyroid extract (Collip) in doses of 20 to 40 units (1 to 2 c.c.) on alternate days.

This type of treatment was continued until January, 1930, when the dose of calcium lactate was raised to 360 grains per day. At this time the symptoms, though reduced, were persistent, and the patient began again to take about 2.5 c.c. of parathyroid extract daily. On March 11, 1930, lactose was added in amounts of 360 grains per day, with two drams each of lactose and calcium lactate before meals. The patient became symptom-free, and the parathyroid extract was reduced gradually.

Many important details have been necessarily omitted, but this outline conveys some idea of the severity of the disease in this case and of the type of treatment employed. Serum calcium and blood phosphate levels are not quoted in detail, but from January, 1925, to March, 1930, the serium calcium varied from 6.2 mg. to 9.7 mg. per 100 c.c., according to

the treatment used. The most constant figure was between 7 and 8 mg. The blood phosphates in January, 1930, before beginning treatment with lactose, were 6 mg. per 100 c.c.

At the time indicated at the beginning of Chart 11 the patient was receiving 1 c.c. of parathyroid extract (Collip) per day, 300 grains of calcium lactate, and 360 grains of lactose. As she had been symptom-free since beginning the use of lactose, it was decided that the calcium lactate and lactose should be stopped entirely in order to see how much parathyroid extract (Collip) was necessary to relieve the symptoms completely. With the patient's permission and cooperation, on April 9 no calcium or lactose was taken at noon or in the evening, two drams of each being taken before breakfast as usual.

Early in the morning of April 10 symptoms appeared and therefore 2 c.c. of parathormone (parathyroid extract, Collip) were given subcutaneously at 8 A.M. and 4 c.c. at

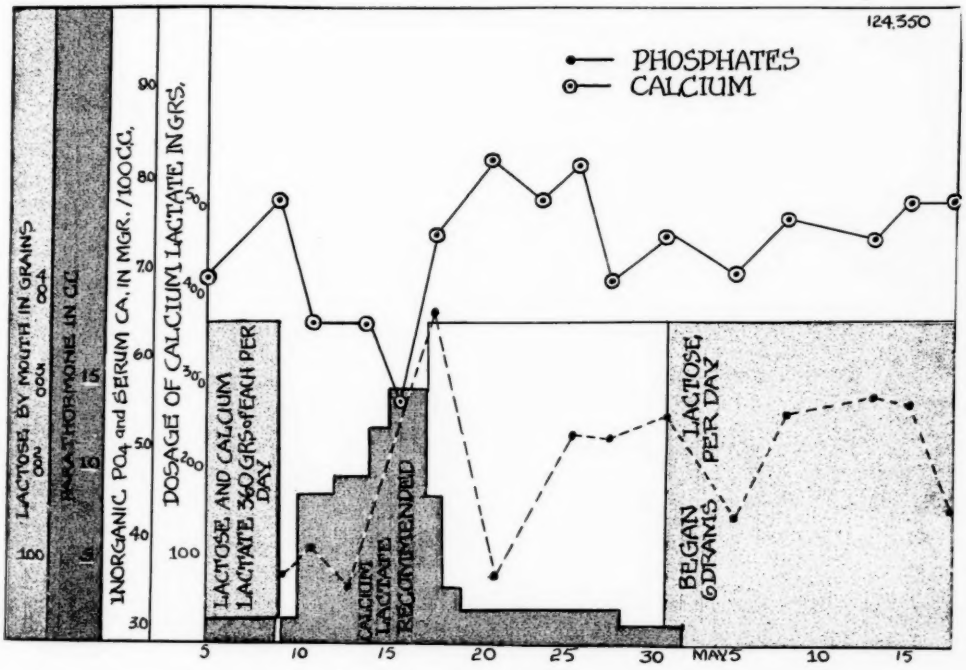
124.350
PHOSPHATES
CALCIUM
LACTOSE BY MOUTH IN GRAINS
PARATHORMONE IN C.C.
INORGANIC PO4 and SERUM CA. IN MGR. /100 C.C.
DOSAGE OF CALCIUM LACTATE IN GRS.
LACTOSE AND CALCIUM LACTATE 360 GRS. of EACH PER DAY
CALCIUM LACTATE RECOMMENDED
BEGAN 6 DRAMS LACTOSE PER DAY
MAYS

Chart 11

5 P.M. On April 11 the severity of the symptoms increased and 8 c.c. of parathormone were used, 4 c.c. at 8 A.M. and 4 c.c. at 5 P.M. The same dose was given on the following day. The blood calcium apparently was falling, but the phosphates were being held well in check. On April 13, despite the administration of 4 c.c. of parathormone at 8 A.M. and 5 c.c. at 5 P.M. the symptoms were becoming more severe. The fingers were stiff, the patient was nauseated all day and took only small amounts of food with difficulty, and she staggered markedly when she walked. On April 14, 6 c.c. of parathormone were given subcutaneously at 9 A.M. There was slight relief in half an hour, but at 4:30 P.M. the symptoms again became more severe. On April 15, 6 c.c. of parathormone were injected at 8 A.M. and 7 c.c. at 5 P.M. The patient was slightly better on this day, but still had some carpal spasm and nausea. On April 16, the symptoms were severe. The muscles of the arms and legs were becoming sore. Seven c.c. of parathormone were given at 8 A.M. and 6 c.c. at 5 P.M. The report on the blood chemistry showed that the serum calcium had fallen to 5.2 mg. and the phosphate had risen to 4.5 mg. per 100 c.c. Because of this and the severity of the symptoms, calcium lactate was given, 2 drams before dinner and 2 drams at bedtime.

The following day she again began to take calcium lactate, 360 grains per day. The serum calcium rose promptly and the symptoms were completely relieved. The parathormone was reduced to 2 c.c. per day. Since slight symptoms were present, the dosage of parathormone was not decreased until April 28. The symptoms from April 17 to May 1 were very slight, but not mild enough for the parathormone to be discontinued. On May 2, 360 grams of lactose were given, divided into three doses, before meals, in addition to the same dosage of calcium lactate as before. The patient was instructed to use parathormone as before, when required for the relief of symptoms. Up to May 8, she had not found it necessary to use any injections, as there had been no paresthesia or stiffness of the fingers since administration of lactose was started.

It was expected that the phosphate level would rise somewhat after the discontinuance of the parathormone, and this has occurred. The patient has noticed very mild symptoms on two days, but they have been so slight that up to the present time she has preferred not to use parathormone.

The patient was last seen on May 20, when she was practically symptom-free.

DISCUSSION

In chronic tetany, definite improvement in symptoms apparently results from the feeding of lactose. This is associated with a fall in the level of inorganic phosphates in the blood. During glucose assimilation after the ingestion of glucose or other carbohydrates, there is always temporary improvement in symptoms, associated with a fall in the phosphate level. Nevertheless, even frequent carbohydrate feedings do not result in the permanent benefit which is observed after the administration of lactose. After single doses of glucose or lactose the blood phosphate returns to the previous level within four hours. It seems remarkable that when lactose is used therapeutically, low levels of inorganic phosphate can be demonstrated in the blood twelve to fourteen hours after the last dose of lactose is taken.

The complete mechanism of the action of lactose on blood phosphates is obscure. At first it was thought that the slow digestion of lactose, and the resulting slow absorption of glucose and galactose, might account for the prolonged depression in the phosphate level in the blood. After examination of the blood following the oral administration of one dose of lactose this seems unlikely. The blood sugar level rises sharply and falls again within four hours, indicating rapid absorption and assimilation. The inorganic phosphates also return to a normal level within this period. The feces have not been examined quantitatively, but it has been shown that the phosphate depression is not the result of increased urinary excretion. On the contrary, there is a retention of urinary phosphates after the administration of single doses of lactose.

The possibility that galactose might have a specific effect was considered. It is known that glucose can be formed in the body from galactose which has been absorbed from the intestine. If the glucose, so formed, is assimilated in the same manner as ingested glucose, it was thought that an extended period of carbohydrate assimilation might result. This in turn would cause a prolonged depression of the phosphate level which would simulate the long, low phosphate curve noted after the administration of glucose to diabetics, when assimilation of the glucose is delayed.

At present, the data concerning this point are insufficient to warrant definite conclusions. Our results, however, and those of Barrenscheen[25] fail to demon-

strate any connection between the metabolism of galactose and that of inorganic phosphate in the blood. The use of galactose over long periods may cause us to draw different conclusions. As yet, there is no definite evidence that the efficacy of lactose is due to changes in intermediary metabolic processes. It may be that there is a slowing of the absorption or an acceleration of the excretion of the phosphates.

Neither Dragstedt[17] nor his recent supporter, Hutton,[27] have associated the effect of lactose with mineral metabolism. Those who believed that parathyroid tetany was caused by changes in calcium metabolism were of the opinion that lactose produced an increased absorption of calcium by the production of acidity in the gut.[26] So far as we are aware, no one has demonstrated an increased blood calcium after lactose administration. Since calcium phosphate is very insoluble, it is possible that an increased absorption of calcium might result in a lowering of the phosphate level due to a deposition of calcium phosphate in the tissues. Hence, increased calcium absorption might not be apparent on examination of the blood.

SUMMARY AND CONCLUSIONS

1. The symptoms of chronic parathyroid tetany may be lessened in severity or completely controlled by lowering of the amount of inorganic phosphates in the blood without raising the total calcium content of the blood serum.

2. Glucose temporarily lowers the amount of inorganic phosphates in the blood of normal subjects and in individuals with chronic parathyroid tetany.

3. Lactose in single doses has the same effect as glucose.

4. Galactose in single doses has no effect on the inorganic phosphate content of the blood.

5. After ingestion of glucose or of lactose there is a decrease in the amount of phosphate excreted in the urine. This does not occur after the administration of galactose.

6. Three cases of chronic parathyroid tetany are reported in which symptoms were present in spite of the oral administration of large doses of calcium lactate. The addition of lactose resulted in complete relief, associated with a lowering of the phosphate content of the blood.

7. One case of chronic parathyroid tetany is reported in which large doses of calcium lactate alone failed to give complete relief and very large doses of parathyroid extract (Collip) alone failed to give relief. The symptoms could be controlled by the oral administration of large doses of calcium lactate, together with the subcutaneous injection of parathyroid extract. Calcium lactate in large doses in combination with lactose gave relief without the addition of parathyroid extract.

8. The mechanism of the action of carbohydrates in lowering the amount of phosphates in the blood is discussed.

We are greatly indebted to Miss Louise Van Alstine for her technical assistance throughout the work.

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A COMPARISON OF THE VASOCONSTRICTIVE ACTION OF ADRENALIN AND EPHEDRINE ADDED TO THE LOCAL ANESTHETIC SOLUTION*

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IN THE introduction of adrenalin, local anesthesia was given, a drug, the local application of which causes a contraction of terminal blood vessels, rendering the tissues bloodless and causing an increase of the local anesthetic action and diminishing the general toxic action. It is, thus, of importance because of its ischemia producing properties. Adrenalin is not an anesthetic agent itself, but local action of other drugs is made more intense and lasting when combined with it. The extent of the anemia of tissues and anesthesia are independent of one another. The first depends upon the adrenalin content, while the latter depends upon the quantity of local anesthetic drug in the solution. Diluted solutions of anesthetic substances can thus be made to produce a more intense effect of longer duration by the addition of adrenalin. The limits of usefulness of local anesthesia have thus been materially increased, the results are more certain, the technic in many instances has been simplified, and danger from certain operations has been markedly reduced.

The dose of adrenalin should be carefully considered, as large doses are very dangerous. The greater the concentration, the greater the toxic action. The chief toxic symptoms are palpitation of the heart, depression, and often a feeling of dread or alarm; difficult respiration, a sense of constriction across the chest, and a fullness and throbbing in the head. These symptoms, accompanied by rapid rise in blood pressure, usually pass off in a few minutes, when the drug should be more cautiously used. Operators often blame the local anesthetic for such symptoms occurring in the course of an operation, when the adrenalin is the disturbing agent.

In the strengths ordinarily employed, its use is not followed by any after reaction. There is no hyperemia, but the tissues gradually resume their normal vascularity, and there seems to be no retarding or injurious action upon the healing of wounds. When used in fairly large doses, it seems that there is often more after pain in the wound than would have been the case under ordinary conditions. If an excessive dose is used, there often follows a primary anemia which is marked and prolonged, a vasomotor paralysis, during which time the vessels remain dilated with open mouths, and secondary hemorrhages may occur.

Following the use of excessive doses, the resulting anemia may be so profound and prolonged as to lead to death of a tissue with sloughing. The author has found that for ordinary surgical work the addition of ten drops to 100 c.c. of anesthetic solution usually suffices. The dose should vary according to the age and condition of the patient. Childhood, old age, arteriosclerotics,

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and those suffering from lesions of the vascular system, high blood pressure, Grave's disease, diabetes, and others, are more susceptible to its influence. The dose in these cases should be lessened accordingly, and often should not be used at all.

For many years adrenalin has been the sole sympathomimetic (i. e., producing effects similar to the result of excitation of sympathetic innervations) drug in clinical use. Recently, its sole competitor, ephedrine, has been introduced. The rise of ephedrine from obscurity to its present state of widespread popularity involves several features of unusual interest. In a short space of time it has found clinical favor in the treatment of bronchial asthma, hay fever, bronchitis, Adams-Stokes syndrome, combatting the fall in blood pressure in spinal anesthesia, shrinking the congested nasal mucous membrane, and in dilating the pupil for ophthalmic examinations.

Ephedrine produces vasoconstriction as determined by perfusion experiments on the frog's leg, the ear of a rabbit, and the intestine, spleen, and kidney of the dog. This action, like that of adrenalin, is essentially peripheral, and not dependent upon stimulation of the vasomotor center or other parts of the central nervous system. Both experimental and clinical data show, however, that as a vasoconstrictor, it is much less powerful and uniform in its effects than adrenalin.

More recently various combinations of adrenalin and ephedrine have found favor in clinical use. Adrenalin applied to mucous membranes has a prompt and powerful vasoconstricting action upon the capillaries reducing congestion and turgescence of the tissues. This action, however, is of relatively short duration. Ephedrine is added to supplement the action of adrenalin, prolonging the astringent effect materially. Hypodermically administered, the advantage is that the action is more lasting than that of adrenalin alone. Schaumaun has found that very small amounts of adrenalin augment the constrictor action of ephedrine in experimental work.

No adequate information seems available on the comparative vasoconstrictive action of adrenalin and ephedrine when added to the local anesthetic solution. This has constituted one of the most important uses of adrenalin and thus far ephedrine has not entered this field of usefulness. Since the systemic action of ephedrine is less intense, it occurred to the author that it might be a more suitable local anesthetic adjuvant in those cases of thyrotoxicosis, hypertension, and cardiopathies, in which adrenalin is poorly tolerated.

The fact that the action of ephedrine is more prolonged than that of adrenalin, also suggests a possible advantage in adding a combination of the two to the local anesthetic solution for a more prolonged local anesthesia. This would thus be similar to the employment of the combination of adrenalin and ephedrine in rhinology, and in hypodermic use. In order to solve this question the following work was carried out:

METHOD

The anesthetic power of all combinations was determined by dermal wheals on the human skin. This is an attractive method because it parallels clinical usage. It involves direct action on the terminal nerve filaments and sensory

end organs of the skin. Anesthesia is but very little dependent upon pressure within the layers of skin, because control wheals of physiologic salt solution do not produce anesthesia. Anesthesia, therefore, results from a direct chemical action upon the nerve endings, which may be prolonged by a vasoconstrictive drug by retarding absorption.

TECHNIC

The anterior surfaces of the thighs were closely shaved. Dermal wheals were then raised with a special local anesthesia syringe and finest hypodermic needle. The needle was thrust beneath the skin surface with bevel downward. At the moment the needle point entered the epidermis, injection began, which was always endermic and not subcutaneous.

The area of wheals was estimated as the size of a dime and required 0.8 to 1 c.c. of solution each. It is important that all wheals be as nearly the same size as possible and contain the same amount of solution, all of which has been injected intracutaneously. Adequate controls were employed, so that no disturbance of sensation except that of complete abolishment of sensation was interpreted as anesthesia.

All wheals were made upon the writer by himself. The skin of the thighs is of such thickness that accurate wheals may be raised painlessly when the substance is anesthetic. The sensitiveness of the skin and the rapidity of absorption vary in different areas of the body. These features also vary in different individuals, depending upon familial traits, exposure, vocation, etc. By employing the same skin area in the same individual, these factors remain constant. The duration of anesthesia in the same cutaneous area may also be shortened by previous brisk massage, heating or muscular exercise because of the improved circulation and consequently more rapid absorption. In these tests the subject remained seated and sources of external heat were avoided. Wheals were marked with a circle of mercurochrome as soon as raised, so that the center of the endermic infiltration was easily identified for testing after the wheal had disappeared. Tests for sensation were made by scratching the area with a wooden applicator or with a needle, as is done in vaccination.

The anesthetic drugs employed were novocaine (Metz) and neothesin (Lilly). Adrenalin chloride solution 1:1000 with chloretone added as a preservative, as furnished to the trade by Parke Davis & Company was used. A supply of c. p. ephedrine hydrochloride was furnished by the Research Laboratories of Eli Lilly & Company especially for these tests. In addition, the 3 per cent ephedrine solution with 0.5 chloretone as a preservative as furnished to the trade by Abbot was available. Chloretone itself possesses local anesthetic properties in 0.5 per cent strength, but not in the high dilution of 10 drops of the stock ephedrine, chloretone solution to 100 c.c. of salt solution.

Table I, first line, shows the anesthetic power of novocaine alone both by duration of anesthesia in minutes and minimal anesthetic concentration. All figures represent the average of many trials. The second line shows the prolongation of anesthesia by the addition of 10 drops of 1:1000 adrenalin solution to 100 c.c. of anesthetic solution. In all these tests the duration was more than twice that of the anesthetic solution alone.

TABLE I

ADRENALIN AND EPHEDRINE

	DURATION OF ANESTHESIA IN MINUTES						
Per cent Strength	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Novocaine alone	23	16	10	6	?	0	0
Novocaine and adrenalin	64	49	30	10	3 ?	0	0
Novocaine and ephedrine	25	21	16	12	4 ?	0	0
Novocaine and adrenalin plus ephedrine	65	52	35	12	3 ?	0	0

Ten drops of 3 per cent ephedrine solution were next added, results of which are shown in the third line. A comparison of these values with those of novocaine alone shows but a slight increase, although the concentration is 30 times that of adrenalin in line two.

The last line shows values for the combination of adrenalin and ephedrine added to the anesthetic solution. It will be observed by comparison with the second line that there is no advantage by the addition of ephedrine in thirty times the amount of adrenalin.

The same series of tests were employed with neothesin (Lilly) as the local anesthetic agent, results of which are expressed in Table II. An inspection of

TABLE II

ADRENALIN AND EPHEDRINE

	DURATION OF ANESTHESIA IN MINUTES						
Per cent Strength	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Neothesin alone	27	24	18	15	9	6	?
Neothesin and adrenalin	63	60	52	30	22	15	6 ?
Neothesin and ephedrine	35	23	20	15	10	7	?
Neothesin and adrenalin plus ephedrine	66	55	41	28	25	12	?

these data corroborates in all respects the facts brought out in Table I. There is no prolongation of local anesthetic action by the addition of ephedrine in thirty times the concentration of adrenalin commonly employed. Neither is there any advantage in the combination of adrenalin and ephedrine as an adjuvant to the local anesthetic solution.

COMMENT

While it is to be admitted that ephedrine has a slight vasoconstrictor action, it is very weak indeed and only serves to emphasize the fact that adrenalin is one of the most powerful drugs known. The results of experimental investigators differ widely. Thus Amatsu and Kubota observed constriction of frogs' legs upon perfusion with a 1 to 10,000 solution of ephedrine hydrochloride. The vessels of the ear of the rabbit were constricted by solutions ranging from 1 to

2000 to 1 in 20,000. Loo and Read working with toads found a 1 in 20,000 solution effective. Other workers while agreeing that ephedrine is a vasoconstrictor, found it a much less powerful one than these results would indicate. Thus Kreitmar found constriction of frogs' vessels with a 1 in 10 dilution of ephedrine, but none with a 1 in 100 solution. Gradinesco found only slight constriction with a 1 in 100 solution. These differences are confusing and seem entirely too great to fall within the realm of experimental error. It was, therefore, decided to titrate the vasoconstrictive power of ephedrine against that of adrenalin, by comparing dilutions of each required to prolong similar strength local anesthetic solutions an equal interval of time. The amount of ephedrine added was thus doubled until equal amounts of 3 per cent strength ephedrine and 1 per cent novocaine were used. In no instance was there prolongation of anesthesia. In fact the higher amounts diminished the duration, indicating an incompatibility when the amount of ephedrine added was too great.

CONCLUSIONS

1. The peripheral vasoconstrictive power of adrenalin is strikingly demonstrated by the prolongation of anesthesia when very small amounts are added to the local anesthetic solution. This action has earned for it the name of "chemical tourniquet."

2. Ephedrine, its only competitor in general clinical use cannot be shown to possess any vasoconstrictive power at all by the dermal wheal method on man.

3. No value can thus be demonstrated by its combination with adrenalin as an adjuvant to the local anesthetic solution.

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STUDIES IN THE SEROLOGY OF SYPHILIS*

VII. On the Supposed Artificial Induction of a Positive Wassermann Reaction in Originally Negative Human Sera

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IT IS commonly believed that the positive Wassermann test observed in syphilis reflects an obscure physicochemical change in the serum colloids, rather than the appearance of a new specific substance. This theory is apparently borne out by the reputed ease of inducing positive Wassermann reactions in originally negative serum by such simple procedures as shaking, acidification, dilution, etc.

As will be shown in the present paper, however, most of the observations which seem to confirm this concept of the Wassermann as a labile and altogether anomalous reaction, are based upon errors of technic and interpretation. Far from being readily induced in normal sera by the most diverse nonspecific processes, it is difficult if not impossible to produce a positive Wassermann in originally negative human serum by such simple physical or chemical treatment.

It will be further shown that the majority of the procedures reputed to induce a positive Wassermann reaction make the serum somewhat anticomplementary. The supposed positive reaction is in reality a summation of two anticomplementary effects, that of the antigen and that induced in the serum by the preliminary treatment. An example will make this clear. The Wassermann reaction actually measures the amount of complement which remains free after the interaction of complement, antigen and serum. If this residual quantity is sufficient to cause hemolysis, the reaction is negative; but if so much has been destroyed that the remaining quantity does not suffice to hemolyze sensitized cells, the reaction is adjudged positive. The exact proportion of complement which must be destroyed in order to obtain a positive reaction varies with the technic used. Let us assume it to be 60 per cent, as it is in a technic using 2 to 2½ units of complement. At the time when most of these artificial positives were reported, the amount of antigen used in the test was so close to the completely anticomplementary quantity that it must have destroyed considerable complement, even though not enough to prevent hemolysis entirely. Thus, Hirschfeld and Klinger (1914) used antigen in one-half of its completely anticomplementary quantity. As is illustrated in Protocol 1 and Table I, the antigen in such a setup may destroy fully half the amount of complement which must disappear in order to give a positive Wassermann reaction; i. e., 25 to 40 per cent in the hypothetical case cited.

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PROTOCOL 1

Dried powdered beef heart (Difco) was extracted for three days with 95 per cent alcohol (5 c.c. per gram). The filtered extract was diluted with 4 volumes of NaCl N/7 (0.85 per cent), and serially decreasing quantities of the dilution incubated with complement, as indicated in the following set-up.

Antigen 1:5, c.c.	0.2	0.1	0.05	0.025	0.0125
NaCl N/7, c.c.	0.2	0.3	0.35	0.375	0.3875
Guinea pig complement, 1:10, c.c.	0.2	0.2	0.2	0.2	0.2

After four hours' incubation at 0° to 6° C., and one-half hour at 37° C., 0.4 c.c. of a sheep cell suspension sensitized with 4 units of rabbit amboceptor were added to each tube, and the time necessary for complete hemolysis was noted. As has been shown elsewhere (Eagle, 1929), the hemolysis time is an accurate measure of the amount of complement destroyed. The results of many experiments, illustrated in Table I, show conclusively that half, and even one-fourth, of the completely anticomplementary quantity of antigen destroys a significant quantity of complement, even though sufficient remains free to allow for hemolysis in half an hour.

TABLE I

SHOWING THAT FRACTIONS OF THE "ANTICOMPLEMENTARY" QUANTITY OF ANTIGEN DESTROY SIGNIFICANT AMOUNTS OF COMPLEMENT

Antigen 1:5 c.c.	0.2	0.15	0.1	0.075	0.05	0.375	0.025	0.0187	0.0125
Hemolysis	0	0	±	±	±	+	+	+	+
% complement destroyed	90	90	70	60	50	35	30	–	–

If now, by shaking, the serum is also made somewhat anticomplementary (e. g., 30 to 40 per cent), the total complement destroyed is sufficient to prevent hemolysis and one has a pseudopositive result. This is not a fixation by a lipoid-reagin *compound*, as is the true positive Wassermann reaction, but a destruction of complement by an anticomplementary serum plus an anticomplementary antigen. This summation of anticomplementary effects is similar to the irreversible destruction caused by immersing the tube in a water-bath at a temperature of 56° C., and quite unlike complement fixation as given by syphilitic serum. The evidence for this contention is given in the following sections.

I

The experimental procedures here listed have been supposed by various investigators to produce a positive Wassermann reaction *in vitro* in originally negative serum: *Shaking* (Hirschfeld and Klinger, 1914); *acidification* (Nathan, 1920; McMeans, 1923); *dilution* (Nathan, 1918; Forssmann, 1921); *adsorption* with a particulate suspension (Hirschfeld and Klinger, 1914); chemical treatment, as by *amino acids* (Bachmann, 1922), *ether* (Forssmann, 1921), and *agar* (Hirschfeld and Klinger, 1914); *aging* (Rabinowitsch, 1914), etc.

Serum from nonsyphilitic human subjects was treated by these methods (Protocol 2) and subsequently tested for its anticomplementary and Wassermann titers (Protocol 3). The results obtained with a single negative serum are summarized in Table II. Qualitatively similar results were obtained with five lots of pooled serum from nonsyphilitic human beings.

PROTOCOL 2

Methods of treating fresh normal human serum preparatory to studying its anticomplementary and complement-fixing properties.

1. *Shaking.*—Serum was diluted 1-10 in (a) water and (b) NaCl N/7 (0.85 per cent) and shaken in a shaking machine at 240 "to-and-fro" movements per minute for five hours. The solution gradually becomes cloudy due to the denaturation of a portion of the serum globulin, particularly in the aqueous dilution.

2. *Acidification.*—HCl N/5 was added to serum until the point of maximal precipitation was reached (P_H 5.0-5.5). After five hours at room temperature, part of the serum was exactly neutralized by the addition of NaOH N/5.

3. *Dilution.*—Serum was diluted 1:10 with H_2O and allowed to stand for five hours at room temperature.

4. *Adsorption with a particulate suspension.*—(a) Kaolin was added to normal serum, the mixture shaken for one minute, and after standing for one hour at room temperature, centrifuged. Serum was similarly treated with normal and specifically agglutinated suspensions of B. typhosus, B. coli, Pneumococcus I, and sheep red cells, and with the precipitate obtained by adding sheep plasma to a rabbit antisheep serum.

5. *Glycocoll, ether and agar.*—Serially increasing quantities of these substances were added to normal serum, and the mixtures allowed to stand for four hours at room temperatures. The ether-serum mixtures were tested both by layering the ether onto the serum and allowing it to evaporate, as well as by thorough emulsification of the mixture.

6. *Aging.*—Sterile negative serum was kept at icebox temperature for one, two and four months.

PROTOCOL 3

Method used to test the anticomplementary action of the treated sera and their behaviour in the Wassermann reaction.

Anticomplementary Titer.—The sera treated as described in Protocol 2 were inactivated at 56° C. for twenty minutes. To varying quantities of the treated sera in a total volume of 0.8 c.c. were added 0.4 c.c. of 1:10 guinea pig complement, and the mixture placed at 4° to

TABLE II

TREATMENT OF SERUM	C.C. SERUM USED	PER CENT COMPLEMENT DESTROYED BY		
		SERUM ALONE (ANTICOMPLEMENTARY)	SERUM + ANTIGEN (ANTICOMPLEMENTARY + FIXATION)	SPECIFIC INTERACTION OF SERUM WITH ANTIGEN (WASSERMANN)
Original serum	0.4	25	25	0
	0.2	<10	<10	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with NaCl N/7	0.4	65	60	0
	0.2	50	50	0
	0.1	30	35	0
	0.05	15	15	0
	0.025	<10	<10	0
Shaken with H_2O	0.4	>90	>90	–
	0.2	>90	>90	–
	0.1	90	>90	–
	0.05	75	80	0
	0.025	45	30	0
Acidified	0.4	>90	>90	–
	0.2	>90	>90	–
	0.1	70	70	0
	0.05	40	40	0
	0.025	20	20	0

TABLE II (Continued)

TREATMENT OF SERUM	C.C. SERUM USED	PER CENT COMPLEMENT DESTROYED BY: SERUM ALONE (ANTICOMPLEMENTARY)	SERUM + ANTIGEN (ANTICOMPLEMENTARY + FIXATION)	SPECIFIC INTERACTION OF SERUM WITH ANTIGEN (WASSERMANN)
Acidified and Neutralized	0.4	55	60	0
	0.2	35	40	0
	0.1	20	20	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Dilution	0.4	85	80	0
	0.2	55	50	0
	0.1	30	30	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with Kaolin	0.4	30	30	0
	0.2	15	15	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with killed B. Typhosus	0.4	20	20	0
	0.2	<10	<10	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with agglutinated B. Coli	0.4	90	90	0
	0.2	75	80	0
	0.1	60	60	0
	0.05	55	50	0
	0.025	30	30	0
Shaken with sheep-anti-sheep serum ppt.	0.4	>90	>90	–
	0.2	>90	>90	–
	0.1	70	75	0
	0.05	60	60	0
	0.025	40	40	0
Shaken with thick sheep cell suspension*	0.4	20	20	0
	0.2	<10	<10	0
	0.1	<10	<10	0
	0.05	<10	<10	0
	0.025	<10	<10	0
Shaken with agglutinated* sheep cells	0.4	>90	>90	–
	0.2	>90	>90	–
	0.1	85	80	0
	0.05	60	50	0
	0.025	40	30	0

In a similar manner, aged serum, and serum treated with glycocoll, ether, or agar gives exactly the same amount of complement fixation without antigen as it gives with antigen; the serum is Wassermann negative.

A syphilitic serum	0.4	25	>90	>90
	0.2	<10	>90	>90
	0.1	0	>90	>90
	0.05	0	>90	>90
	0.025	0	>90	>90
	0.0125	0	>90	>90
	0.006	0	>90	>90
	0.003	0	70	70

*Heated serum was shaken with the sheep cell suspensions, in order to prevent any hemolysis due to native amboceptor and native complement.

8° C. for four hours, followed by one-half hour at 37° C. Eight-tenths c.c. of sheep cells sensitized with four units of amboceptor were then added, and the time required for complete hemolysis was noted. As has been shown elsewhere (Eagle, 1929), the amount of complement remaining free, and therefore the amount destroyed, can be accurately calculated by measuring this hemolysis time.

Wassermann Titer.—Simultaneously, a quantitative Wassermann reaction, i. e., the determination of the amount of complement fixed and destroyed by the serum in the presence of beef heart lipoid (antigen) was carried out, using the same amounts of complement and the same amount of sensitized sheep cells, in the same total volume under the same conditions. Care was taken to use an antigen which is not in the slightest anticomplementary in the quantity used (0.4 c.c. of a 1:60 dilution in NaCl N/7 of a beef heart antigen containing 0.6 per cent cholesterol; anticomplementary unit 1:5 dilution). The results are summarized in Table II. At the bottom of the table the results obtained with a known syphilitic serum are given for contrast.

Comparing the results obtained by these two series of tests, it is found that the amount of complement destroyed by all the treated sera in the presence of beef heart lipoid is, within the limit of experimental error, the same as is destroyed by the treated sera alone; they are completely Wassermann negative. The various manipulations have succeeded only in making the serum somewhat anticomplementary.

II. PSEUDOPOSITIVE WASSERMANN REACTIONS WITH NONSPECIFIC ANTIGENS

If now, using these more or less anticomplementary sera, one tries complement fixation with a somewhat anticomplementary antigen, the summation of complement destruction by serum and by antigen may, and sometimes does, simulate the phenomenon of complement fixation as given by syphilitic serum. Actually, however, there has been no complement fixation by a reagin-antigen compound, such as determines the positive Wassermann reaction. Substances other than a Wassermann antigen will produce the same pseudoreaction; e. g., weakly anticomplementary suspensions of lecithin, of milk lipoid, of sheep cell lipoid, of cholesterol, or a weak solution of HCl, none of which has the slightest specific reactivity with human syphilitic serum, serve equally well to give this pseudo-fixation of complement (Protocol 4 and Table III).

PROTOCOL 4

The preparation of lipoid "antigens" possessing no specific reactivity with syphilitic serum.

1. Lecithin (Merck) was dissolved in 95 per cent alcohol to form a 2 per cent solution: this was fortified with 0.6 per cent cholesterol.

2. Skimmed milk powder was extracted twice with ether (4 c.c. per gram): the dry residue was extracted with 95 per cent alcohol for three days. The alcoholic extract was then fortified with 0.6 per cent cholesterol.

3. Citrated sheep's blood was washed in 10 volumes of NaCl N/7. The sedimented cells were dried at 60° to 90° C. in a current of dry air not quite to dryness, and the semisolid mass was extracted with alcohol (5 c.c. per gram). The yellow extract was fortified with 0.6 per cent cholesterol.

4. Human blood clots were similarly treated.

5. Cholesterol was dissolved in alcohol as a 0.6 per cent solution. None of these 5 antigens have the slightest selective reactivity with syphilitic serum.

The preparation of a highly anticomplementary Wassermann antigen.

1. Beef heart powder (Difco) was extracted with 95 per cent alcohol (5 c.c. per gram)

for four days. The extract was fortified with 0.6 per cent cholesterol and 0.4 per cent sitosterol. Such an extract, although very sensitive, is highly anticomplementary.

The preparation of a sensitive Wassermann antigen with practically no anticomplementary properties.

Beef heart powder (Difco) was extracted from four times with ether (4 c.c. per gram) at 37° C., each extraction lasting fifteen minutes. The ether extracts, containing >95 per cent of the anticomplementary factors, were discarded, and the dried residue extracted with 95 per cent alcohol. The alcoholic extract was concentrated to one-half its original volume, and sensitized as described in the preceding paragraph. Such an antigen is only slightly more anticomplementary than pure alcohol.

Complement fixation by these "antigens" and chemically or physically altered negative serum.

To normal human serum treated as described in Protocol 2 were added complement and various quantities of the antigens described in the preceding section. The amount of complement destroyed by (1) the serum alone, (2) the antigen alone, and (3) by a mixture of the serum and the antigen was then determined by the method described in Protocol 1 after four

TABLE III

PSEUDOPOSITIVE COMPLEMENT FIXATION BETWEEN THE PARTIALLY ANTICOMPLEMENTARY TREATED SERA AND PARTIALLY ANTICOMPLEMENTARY NONSPECIFIC ANTIGENS

		ANTICOMPLEMENTARY TITRE (NO ANTIGEN)		PSEUDOCOMPLEMENT FIXATION WITH LECITHIN AS ANTIGEN					
				1:8000		1:4000		1:2000	
		% COMP. DESTROYED BY SERUM ALONE	READING OF FIXATION	% COMP. DESTROYED	READING OF FIXATION	% COMP. DESTROYED	READING OF FIXATION	% COMP. DESTROYED	READING OF FIXATION
Serum diluted and shaken with H_2O c.c.	0.4	>90	+	>90	+	>90	+	>90	+
	0.2	90	+	>90	+	>90	+	>90	+
	0.1	70	±	75	+	>90	+	>90	+
	0.05	30	0	30	0	80	+	>90	+
	0.025	15	0	20	0	60	0	90	+
	0.0125	–	0	–	0	55	0	80	+
	0.0062	–	0	–	0	40	0	70	±
	0.0031	–	0	–	0	45	0	70	±
	0 (antigen alone)	–	0	–	0	40	0	70	±
Normal serum shaken with agglutinated staphylococci	0.4	>90	+	85	+	>90	+	>90	+
	0.2	75	±	75	±	>90	+	>90	+
	0.1	70	±	70	±	90	+	>90	+
	0.05	60	0	60	0	75	±	>90	+
	0.025	30	0	50	0	60	0	90	+
	0.0125	15	0	25	0	60	0	75	±
	0.0062	–	0	–	0	50	0	65	±
	0.0031	–	0	–	0	45	0	65	±
	0 (antigen alone)			–	0	50	0	65	±
Syphilitic serum	0.4	40	0	40	0	70	0	80	+
	0.1	–	0	–	0	40	0	70	±
	0.01	–	0	–	0	40	0	70	±
	0.005	–	0	–	0	40	0	70	±
	0.001	–	0	–	0	40	0	70	±
	0.0001	–	0	–	0	40	0	70	±
	0 (antigen alone)	–	0	–	0	40	0	70	±

+ Indicates complete fixation; no hemolysis.
± Indicates incomplete fixation; partial hemolysis.
– Indicates complete hemolysis.

hours at 4° to 8° C. and one-half hour at 37° C. The results obtained with two types of treated serum, with lecithin as antigen are given in Table III: qualitatively similar results were obtained with each of the others.

As is illustrated in Table III, the amount of complement destroyed by mixtures of serum and any of these "antigens" is simply the sum of the amount destroyed by each alone. There is in no case true complement fixation such as is given by a mixture of syphilitic serum and beef heart lipoid. One of these "antigens" may be partially anticomplementary in the quantity used and yet allow hemolysis to take place in half an hour (a "negative" antigen control). Similarly, a physically or chemically altered serum may be partially anticomplementary and nevertheless allow hemolysis in the quantity used. Yet the same quantities of serum and of "antigen" when combined may destroy enough complement to prevent hemolysis in half an hour and give a reaction simulating the specific positive Wassermann. It is interesting to note that the anticomplementary extract of beef heart gave this type of false positive reaction with the sera altered by the above mentioned treatment, while an antigen prepared from the same lot of beef heart and possessing just as great reactivity with syphilitic serum, but without the anticomplementary properties of the other antigen, gave completely negative results.

One can readily understand why these artificially "positive" sera fail to give the precipitation reactions for syphilis (Nathan 1920); for these tests predicate an actual combination between antigen lipoids and syphilitic antibody, and merely making a serum anticomplementary would not affect a reaction in which no complement is used.

III. THE COMPLEMENT FIXING PROPERTIES OF GLOBULIN DERIVED FROM FRESH SERUM

We have found only one procedure which induces a true positive Wassermann reaction in originally negative human serum. This is the precipitation of globulin from fresh serum as reported by Forssman (1921). Redissolved in NaCl N/7, this precipitate gives weak but definitely positive complement fixation with beef heart lipoids. This observation, however, does not prove the anomalous nature of the Wassermann substance (reagin), because unlike syphilitic serum, the globulin solution gives complement fixation with almost any finely dispersed lipoidal suspension. As is shown in Table IV, such a globulin solution reacts strongly with an alcoholic extract of sheep or human red cells, of milk, and with an alcoholic solution of lecithin and cholesterol, none of which reacts specificially with syphilitic serum.

PROTOCOL 5

The reactivity of globulin derived from normal fresh human serum with diverse lipoidal suspensions.

To 5 c.c. of fresh Wassermann-negative human serum were added 45 c.c. of cold H_2O; CO_2 gas was bubbled through the dilution for five minutes. The turbid suspension was then centrifuged, the supernatant fluid discarded, and the globulin precipitate redissolved in 5 c.c. of NaCl N/7. The solution was then tested for (1) anticomplementary titer, and (2) complement fixing reactivity by the technic described in Protocol 3, using the antigens described in Protocol 4. Care was taken to use a dilution of the antigen which is not demonstrably anticomplementary (⅛ of the anticomplementary quantity). The results are summarized in Table IV.

TABLE IV

REACTIVITY OF THE FRESH NORMAL HUMAN GLOBULIN WITH VARIOUS LIPOIDAL SUSPENSIONS

GLOBULIN SOLUTION C.C.	ANTICOMPLEMENTARY TITER (NO ANTIGEN)		CHOLESTEROLIZED ALCOHOL EXTRACT OF BEEF LIPOID 1:40		CHOLESTEROL 1% SOLUTION 1:400		LECITHIN 3% SOLUTION 1:8000		CHOLESTEROLIZED MILK EXTRACT		CHOLESTEROLIZED ALCOHOLIC SHEEP RED CELL EXTRACT 1:800		CHOLESTEROLIZED ALCOHOLIC HUMAN RED CELL EXTRACT 1:400	
	% COMP. FIXED	READING OF FIXATION	% COMP. FIXED	READING OF FIXATION	% COMP. FIXED	READING OF FIXATION	% COMP. FIXED	READING OF FIXATION	% COMP. FIXED	READING	% COMP. FIXED	READING	% COMP. FIXED	READING
0.4	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.2	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.1	90	+	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.05	50	0	>90	+	>90	+	>90	+	>90	+	>90	+	>90	+
0.025	25	0	>90	+	90	+	>90	+	>90	+	>90	+	>90	+
0.0125			>90	+	40	0	90	+	90	+	>90	+	>75	+
0.0062			90	+	20	0	70	±	50	0	>90	+	40	0
0.0031			60	±	–	0	35	0		0	90	+	0	0
0.0016			30	0	–	0	–	0		0	65	±	0	0
Approximate Titer in Complement Fixing Units per c.c.			200		30		160		120		480		80	
Titer of a strongly positive syphilitic serum with these antigens			500		0		0		0		0		0	

+Indicates complete fixation; no hemolysis.
±Indicates incomplete fixation; partial hemolysis.
–Indicates complete hemolysis.

The globulin solution thus contains protein which reacts with apparently any properly dispersed lipoid. It can therefore not be considered to represent artificially created Wassermann reagin, which reacts with a certain particular tissue lipoid, and not with cholesterol, milk lipoid, red cells lipoid, lecithin, etc.

SUMMARY AND DISCUSSION

We have been unable to make an originally negative human serum Wassermann positive by physical or chemical treatment. As described in the text, most of the manipulations reputed to induce such an artificial positive reaction succeed only in making the serum anticomplementary. It is true that the globulins precipitated from fresh sera are Wassermann positive insofar as they give complement fixation with beef heart lipoid; but unlike syphilitic serum, this globulin solution reacts equally well with milk lipoid and egg lecithin, with cholesterol and an alcoholic extract of sheep or human red cells, and presumably, with any colloidal lipoid suspension. None of the other methods succeed even to this extent.

The experiments purporting to prove that the positive Wassermann reaction of syphilitic serum is due to a labile physicochemical change in the serum colloids, readily duplicated in vitro, have therefore not been confirmed.

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STUDIES IN THE SEROLOGY OF SYPHILIS

VIII. A New Flocculation Test for the Serum Diagnosis of Syphilis*

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WITHIN the last ten years there has been an increasingly widespread use of various precipitation tests for syphilis. Their simplicity contrasts sharply with the laborious complexity of the Wassermann reaction; and although their value as compared with that of the Wassermann is still controversial, they undoubtedly represent a very significant advance in the serologic diagnosis of syphilis.

It is perhaps unfortunate that so many of these tests have been devised. In principle they are all identical; and only superficial variations in technic distinguish most of them. In the face of the recognized value of the Kahn test, the Meinicke-Klärungs-reaction, the Müller-Ballungs-reaction, and several others, it would seem useless to add still another test to an already confusing array, were it not that its sensitivity, technical simplicity, and ease of reading, all made possible by the use of a new sensitizing substance, are difficult to reproduce in any of the tests now available.

I. The use of corn germ sterol as a sensitizing substance

In the precipitation tests for syphilis, the "antigen" is an alcoholic extract of animal tissue, usually, beef heart. When diluted with saline, this extract forms a milky suspension of lipoid particles which remain stable when added to normal serum, but agglutinate in syphilitic serum to form visible aggregates.

It is known that the addition of cholesterol to the alcoholic tissue extract gives rise to a more opaque fluid when the extract is diluted with salt solution, this increased opacity being due to the formation of a larger number of microscopically visible particles. Elsewhere I have brought forward evidence to support the view that these particles consist of a core of cholesterol covered with a superficial film of lipoid which constitutes the reacting surface. The larger particles so formed are, for obvious reasons, easier to agglutinate into visible clumps. Moreover, such "complex" lipoid cholesterol particles have, for an unknown reason, a greater tendency to combine with reagin, explaining the greater efficiency of the cholesterolized antigen in the Wassermann reaction also.†

According to the foregoing conception, the sensitizing action of cholesterol is due solely to its physical properties. If this is true, one might predict that any substance with similar physical properties would have a similar action;

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furthermore, since the degree of sensitization increases directly with the amount of cholesterol, it might be predicted that the discovery of such substances would allow the preparation of much more sensitive antigens for both the Wassermann and precipitation tests than have hitherto been available.

These expectations have been realized in the discovery of a group of alcohol-soluble, water-soluble substances which, on addition to the alcoholic tissue extract, increase its sensitivity in exactly the same manner as does cholesterol. The use of one of these, sitosterol, to produce a more sensitive Wassermann antigen has been described in detail elsewhere.* Since the publication of that paper, another sterol derived from corn germ has been obtained through the cooperation of the Difco laboratories. The physical properties of this substance make it particularly fitted for use in a precipitation test.

When a cholesterolized antigen† is diluted with approximately two volumes of saline, it forms a highly unstable suspension consisting of coarse visible aggregates of particles. The evidence outlined in the first paper of this series indicated that the elements of these aggregates are tiny amorphous particles of cholesterol covered by the active lipoid. If one adds corn germ sterol instead of cholesterol to the beef heart extract, and dilutes as before with two volumes of saline, one obtains a somewhat more stable suspension, microscopically similar to that formed by a cholesterolized antigen. When this suspension is examined microscopically, however, its opacity is seen to be due to myriads of tiny needle crystals, instead of the amorphous particles formed by a cholesterolized antigen. When a small portion of the suspension is added to normal human serum, the crystals remain discrete, and the mixture appears homogenous and diffusely opaque. When shaken, the cloud of refractile discrete crystals is readily visible. In syphilitic serum, however, the crystals rapidly aggregate to form coarse clumps.

II. PREPARATION OF THE ANTIGEN

For the basic alcoholic extract, dry powdered beef heart (100 grams) is extracted three times with four volumes of pure anesthesia ether (400 c.c.) at 37° C., each extraction lasting ten minutes with frequent shaking. This serves to remove unstable substances (fats, soaps, sterols, etc). After the third filtration,‡ the moist powder is washed on the filter paper with one volume of ether (100 c.c.), dried thoroughly, and extracted for three days with 5 volumes of 95 per cent ethyl alcohol. The ether extracts are discarded. The alcohol extract is filtered, and the most powder washed with fresh alcohol until the washings are colorless. The combined alcoholic filtrate and washings (about 650 c.c.) are then evaporated on the steam bath to a volume corresponding to 5 c.c. per gram of original powder (500 c.c.). The lipoid content of the final extract is usually around 1.6 per cent. Six-tenths per cent cholesterol and 0.6 per cent corn germ sterol are then added and dissolved by boiling to form the stock antigen. Kept in the icebox, it is good for at least twelve months.

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*J. Exp. Med. **53**: 605, 1931.

This antigen is used in a 1:200 dilution, prepared by dropping the antigen slowly with shaking into 200 volumes of 0.85% NaCl. This has been found to be a more satisfactory method of dilution than that recommended in the paper cited.

†Tissue lipoids 1½ per cent; cholesterol 0.6 per cent.

‡With suction, to prevent undue evaporation.

III. TECHNIC OF THE TEST*

The antigen is heated at 65° to 85° C. for a few minutes to dissolve the excess sterol, and 1.3 volumes of 4 per cent NaCl rapidly blown into 1 volume of the antigen. This antigen dilution should be allowed to "ripen" for at least half an hour. Unlike the Kahn antigen, this dilution need not be used within a certain short period of time. If kept in the icebox, it can be used as long as 3-6 days after its preparation. Because its sensitivity gradually increases as it ages, the routine procedure in this laboratory is to prepare the dilution one day in advance, allowing it to ripen in the icebox for twenty-four hours before use.

The serum to be tested is inactivated for twenty to thirty minutes at 56° C., and 1/8-1/12 its volume of the antigen dilution added. If the antigen suspension is older than three days, the amount added to the serum should be decreased to 1/15 the serum volume. Although the test can be carried out with 0.2 c.c. of serum and 0.02 c.c. of antigen dilution, it is more convenient to use twice these quantities. The turbid mixture is then shaken for two minutes. The incubation period can be adjusted at will to suit the circumstances.

If a rapid reading is necessary, as for an emergency transfusion, the tube is incubated at 37° C. for one-half hour. Otherwise, it may be incubated at 37° C. for 4 to 8 hours. It need hardly be added that the longer the incubation period, the more sensitive are the results obtained. Our routine procedure is to incubate for 4 hours at 37° C. In any event, after incubation, the tube is centrifuged at 1500 r.p.m. for 10 to 15 minutes. Three volumes of NaCl N/7 (0.85 per cent) are then added; i. e., three times the volume of serum used.

In a negative reaction the tube is seen to be homogeneous and diffusely opalescent. On shaking one sees a cloud of tiny refractile crystals, not visible if the tube is at rest. In a positive reaction these crystals clump to form coherent coarse white floccules floating in a clear and transparent fluid. There is the sharpest possible contrast between the water-clear fluid and coarse floccules of the positive test, and the homogeneous opacity of the negative test.

One occasionally encounters weak positives, particularly in patients under antisyphilitic treatment. Usually, aggregation in such cases is definite; but even when there is only a slight granular appearance, resembling a positive Kahn, not sufficiently marked to justify a definite reading of *positive*, a second centrifugation at higher speed usually enables one to evaluate the results in terms of positive or negative. If aggregates are really present, they are thrown down to form a coherent floccule at the bottom of the tube, covered by a clear supernatant fluid. In a negative, the crystals remain discrete and are not thrown down: the tube remains homogeneous and opalescent.

Although the macroscopic reading is far more satisfactory, it is possible to read the results by microscopic examination. In a negative test one sees myriads of tiny crystals which do not cohere even though they are in immediate contact. In a positive test these crystals are clumped in much the same manner that red cells are clumped by an agglutinating serum, leaving clear spaces between the aggregates.

*The principles determining the choice of quantities, conditions, etc., will be discussed in a forthcoming paper dealing with precipitation reactions in general (Am. J. Syph., April, 1932).

It is strongly urged that laboratories using the test restrict themselves to the following terms in reporting results: *positive, negative,* and *doubtful,* the latter calling for a repeat test.

IV. ADVANTAGES OF THE TEST

The points of superiority of this test as compared with current serologic methods are briefly enumerated below. The statistical evidence on which the first two points are based will be presented in a later paper.

1. *Sensitivity.*—The reaction is very sensitive, distinctly superior in this respect both to the immediate Kahn reaction, which is acknowledged to be among the best of the flocculation tests for syphilis, and to a four-hour icebox Wassermann test with a sensitive antigen. It detected >70 per cent of a known syphilitic population under specific treatment. The Kahn reaction on the same group of cases yields about 60 per cent positive reactions. These results are to be compared with those obtained in the 1928 League of Nations Conference, at which the best tests detected around 60 per cent of a known syphilitic population.

2. *Specificity.*—The results obtained in testing a general dispensary population indicate that the incidence of false positive reactions is less than ¼ per cent, as judged by the history, clinical signs, Wassermann reaction, or a combination of any or all of these criteria. In a control series of 190 medical students, there was not a single positive reaction.

3. *Simplicity of Preparation of Antigen.*—This is sufficiently obvious from Section I.

4. *Simplicity of Technic.*—Only one tube is necessary, and but 0.2-0.4 c.c. of clear serum. No chemicals are necessary save salt solution, and no apparatus save pipettes, water-baths, and centrifuge.

5. *Ease of Reading.*—The results are so clear-cut as to reduce to a minimum the factor of interpretation. One need not scrutinize the tube very closely for tiny aggregates, as in the Kahn test; when positive, the flocculation is apparent and requires no trained technician for its reading. Occasional doubtful results can be usually resolved into a definite positive or a definite negative by a second centrifugation at higher speed.

6. Cheapness: The most expensive ingredient is the most essential one, the corn germ sterol. Despite the present high cost of this substance, the cost per test is only a fraction of a cent. One c.c. of antigen makes 2.3 c.c. of the final antigen dilution, sufficient for either 50 or 100 tests, depending upon whether 0.2 or 0.4 c.c. of serum are used.

7. It is suitable for immediate tests, as for an emergency transfusion, in which case results can be read within an hour after the blood has been obtained, as well as for a highly sensitive four-hour or overnight test.

8. The antigen dilution keeps for at least three days if stored in the icebox; indeed, the longer it stands, the more sensitive it becomes. Our routine procedure is to dilute the antigen with saline a day before it is used.

9. The test is not so finely adjusted that the slightest variation in technic will make for false negative or false positive results. Surprisingly large variations in the amount of saline used in diluting the antigen (1.1-2 c.c.), in the

age of the antigen dilution (one-half hour to three days), in the proportion of antigen dilution to serum (1:4-1:16), and in the time of incubation (one-half hour to 24 hours), do not appreciably affect the results obtained.

10. Quantitative Test: A quantitative test is readily performed, by determining the dilution in which a serum will still give a positive reaction, as illustrated in the following set-up.

c.c. serum	0.4	0.2	0.1	0.05				
serum diluted 1:16, c.c.					0.4	0.2	0.1	0.05
NaCl N/7	0	0.2	0.3	0.35	0	0.2	0.3	0.35
Antigen dilution, c.c.	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Reading	+	+	+	+	+	+	±	−

The serum is positive up to a 1:32 dilution; i. e., contains 32 units.

SUMMARY

A flocculation test for syphilis is described, based upon the use of a beef heart extract sensitized with both cholesterol and corn germ sterol. Dried powdered beef heart (100 g.) is extracted three times with four volumes of anesthesia ether, each extraction for ten minutes at 37° C. The dried residue is extracted for three days with five volumes of 95 per cent ethyl alcohol. After filtration, the powder is washed with 95 per cent alcohol, and the combined filtrate and washings evaporated down to 500 c.c. Cholesterol and corn germ sterol* are then added, each to a final concentration of 0.6 per cent (600 mg. for 100 c.c. of antigen).

A measured volume of the finished antigen is diluted with 1.3 volumes of 4 per cent NaCl. After ripening for at least half an hour (preferably overnight), 0.04 c.c. of the suspension is added to 0.4 c.c. of inactivated serum (i.e., previously heated at 56° C. for thirty minutes), the tube shaken for two minutes, and incubated at 37° C. for four hours. It is then centrifuged at >1500 r.p.m. for ten to fifteen minutes, 1.2 c.c. of 0.85 per cent NaCl are added and results read.

The reaction is sensitive to a degree (>70 per cent of known syphilities), highly specific (<¼ per cent false positives), and simple to carry out. The results are clear-cut, a negative serum appearing homogeneous and diffusely opalescent, and a positive serum yielding a clear fluid with coarse coherent floccules. The several points of advantage of this test are enumerated in the text. Statistical results in more than 25,000 tests will appear in a following paper.

It should be pointed out that the test as described in this paper is not definitive. The discovery of other sensitizing substances which can be used to replace or supplement cholesterol and corn germ sterol may well make for an increased sensitivity and clarity without endangering the specificity of the results.

*Prepared by Digestive Ferments Co., Detroit.

LABORATORY METHODS

THE VERNES-BRICQ-YVON PHOTOMETER*

Its Application to Routine Biochemical Work, With Special Reference to the Estimation of Phosphorus in Blood

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THE serologic application of the Vernes Bricq Yvon Photometer and its use for the turbidimetric standardization of vaccines, have given rise to a considerable literature. Very little has been published however on its application to chemical analysis. We have not been able to trace any references in British or American literature. For references to serologic and vaccine work in the English language Adelaide Bayliss[1] and Wansey Bayly et al.[2] can be consulted. For a full description of the apparatus with illustrations, details of serologic technic, and a brief chapter on its application to chemical analysis with microchemical technic, the reader is referred to Leger and Martin.[3]

Our own experience with the instrument has convinced us that it possesses overwhelming advantages for routine biochemical analysis. We now use it exclusively in place of the usual colorimeters and nephelometers.

The photometer is an extremely sensitive instrument for the measurement of color or turbidity in definite mathematical terms. The Introduction into the eyepiece of a monochromatic screen renders matching a simple process and does away with the usual colorimetric error due to optical idiosyncrasies of the individual observer. The devisors of the instrument claim accuracy to within three scale divisions. In this laboratory an experience of two years has shown all our technicians capable of measurements which agree within one division of the scale. In applying the instrument we have found that, for a general concentration of turbidity or color, the ratio of light transmitted to light absorbed, viz., optical density, is constant, providing the conditions of preparation of the solutions are identical. For a further discussion of these conditions, the book referred to above[3] will be found useful.

For the past six years we have been engaged in elaborating methods for the routine analysis of all normal constituents of blood, urine, and feces. The scale of work has necessitated the reexamination of present methods with the object of standardizing technic in such a way as to satisfy the following criteria:

1. The use of minimum amounts of biologic material
2. Simplicity of technic
3. Saving of time and bench space
4. Accuracy

We first tested out the photometer for the analysis of blood urea.[4] Up to this time we had been using a microgravimetric xanthydrol method.[5] This was

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extremely accurate, but necessitated much time and the use of a Sartorius microbalance. A long series of experiments over a six-month period checked by a macrogravimetric method, showed that the photometric method furnished an even higher degree of accuracy than the gravimetric method. The photometric method was also extremely rapid and simple and necessitated the use of only 0.2 c.c. serum.

We then proceeded to try out the instrument for various other routine analyses. We now have two Vernes-Bricq-Yvon photometers in constant use in the laboratory. The following routine analyses are carried out exclusively by photometric methods:

Phosphorus fractions in blood
Phosphorus in urine and feces
Sulphur fractions in blood and urine
Magnesium in blood, urine and feces
Uric acid in blood and urine
Urea nitrogen in blood
Amino nitrogen in blood
Cholesterol in blood
Hemoglobin in blood
Creatine and creatinine in urine
Protein in urine
Calcium in urine

We are working at present on the standardization of further microphotometric methods for the estimations of:

Sodium in blood, urine and feces
Potassium in blood, urine and feces
Chlorine in urine
Sulphur in feces

Of the above methods only the photometric blood urea technic has been published as mentioned above. Recently a microphotometric method for the estimation of blood sulphur fractions has been published.[6] The technic independently worked out by these authors is very similar to our own. Our present technic for the routine analysis of the other constituents of blood, urine and feces, will be published in book form in the near future. In view however of the fact that this instrument has been little or not at all used in the routine biochemical laboratory, we have considered it opportune at this juncture to call attention to its advantages. As an illustration we propose to give the details of a microphotometric system for the analysis of the blood phosphorus fractions. We have also considered it useful to record the experimental work leading up to the choice of our present technic.

METHODS FOR BLOOD PHOSPHORUS ESTIMATION

Previously we have had at our disposal a choice of methods based on the following principles:

1. Volumetric
2. Gravimetric
3. Nephelometric
4. Colorimetric

For routine estimation of the various phosphorus fractions in blood, volumetric and gravimetric methods were found unsuitable. They necessitated the use of too large quantities of blood. We were left with present micromethods which can be divided into two groups:

1. Nephelometric or turbidimetric
2. Colorimetric

TURBIDIMETRIC METHODS FOR PHOSPHORUS ESTIMATION

Existing Methods.—The literature contains several accurate methods based upon the capacity of phosphorus for combining to form a white insoluble strychnine phosphomolybdate.

(a) Embden[7] first utilized this principle in his gravimetric technic.
(b) Myrbäck[8] and Roche[9] precipitated the strychnine salt in nitric acid solution and used a titrimetric endpoint.
(c) Rona and Kleinmann[10] have published the only present method for turbidimetric estimation of phosphorus.

For straightforward phosphorus estimation, Kleinmann's method (as published by Rona and Kleinmann), is to be recommended. The author however specifies that the solution containing phosphorus must be devoid of all organic matter before precipitation can be attempted. This necessitates ashing in the case of blood or trichloracetic acid filtrate, and is therefore not suitable for inorganic phosphorus estimation.

Attempted Photometric Application of Turbidimetric Methods.—Of the above, the only principle which lent itself to a possible photometric application, was that of Myrbäck and Roche. We give details of our technic below.

> Five-tenths c.c. clear plasma are added to 1 c.c. water. This is mixed with 1 c.c. of 20 per cent trichloracetic acid. After standing the precipitate is filtered off through a phosphorus-free filter. Five-tenths c.c. of the clear filtrate is transferred to a small hemolysis tube. Two c.c. of water and 1 c.c. of strychnine nitromolybdate reagent are added. This latter is prepared extemporally by mixing 3 c.c. of a solution of 16.5 per cent ammonium molybdate with 33 per cent strong nitric acid to 1 c.c. of a 1 per cent strychnine solution.
>
> The contents of the hemolysis tube are allowed to stand for twenty minutes. At the end of this period the tube is inverted several times in order to obtain homogeneity. The optical density is then read in the photometer. This optical density is compared on a previously constructed graph relating optical density to concentration.

Experimental Results Using Photometric Application of Turbidimetric Principles.—Using a standard solution of KH_2PO_4 the figures arrived at are shown in Table I.

These figures show that a constant turbidity, i. e., constant variations of optical density, can be arrived at when a pure phosphorus solution is used.

TABLE I

MG. P. IN SOL.	OPTICAL DENSITIES		
0.001	9	10	9
0.002	23	25	23
0.003	33	35	35
0.004	50	54	50
0.005	66	64	66
0.006	85	87	85
0.007	101	100	101
0.008	118	117	117
0.009	135	135	135
0.010	148	152	150

Before applying this method to blood, we endeavored to ascertain the influence of trichloracetic acid on the stability of suspension. Using the proportions of free and neutralized trichloracetic acid demanded by the technic, arrived at the figures shown in Table II.

TABLE II

OPTICAL DENSITIES			
MG. P. IN SOL.	PURE SOL.	SOL. + 0.25 C.C. 20 PER CENT TRICHLORACETIC ACID	SOL. + 0.25 C.C. SOD. TRICHLORACETATE
001	10	7	10
003	35	23	26
005	65	49	57
007	100	97	91
009	135	145	120

These figures show that the addition of the reagent alone interferes with stability of suspension to such an extent as to make the method unreliable. A concomitant series of experiments using blood provided confirmatory evidence. As a standard of comparison we used the orthodox Briggs Colorimetric method mentioned below. This had previously been used as a routine method in this laboratory (Table III).

TABLE III

INORGANIC PHOSPHORUS CONTENT OF PLASMA	
BRIGGS COLORIMETRIC METHOD	PHOTOMETRIC APPLICATION OF ADOPTED TURBIDIMETRIC PRINCIPLE
4.05 mg. % P.	3.94 mg. % P.
4.10 mg. % P.	3.15 mg. % P.
4.10 mg. % P.	3.40 mg. % P.

In view of these findings, the question of suspension stability seemed too complex to warrant further work along these lines. We decided therefore to confine ourselves to a colorimetric technic.

MICROCOLORIMETRIC METHODS FOR PHOSPHORUS ESTIMATION

Existing Methods.—The literature contains a large number of accurate microcolorimetric methods. The more important of these can be tabulated as follows:

a. Bell and Doisy[11] first suggested the use of molybdic acid in order to produce a blue color.
b. Briggs[12] modified the above using hydroquinone bisulphite.
c. Benedict and Theis[13] incorporated minor improvements in technic.
d. Kay and Robinson[14] } Variations of the
e. Maitland and Robison[15] } same
f. Stanford and Wheatley[16] } principle.
g. Deniges[17] put forward the principle of the reduction of phosphomolybdic acid with stannous chloride.
h. Kuttner and Cohen[18] carefully investigated Deniges' principle, and showed that definite limits of reacting substances gave specific reduction for phosphorus, although both arsenic and silica might be present in the solution in large amounts.
i. Youngburg and Youngburg[19] confirmed this work and elaborated a system for the microanalyses of the various phosphorus fractions in blood.

j. Fiske and Subarrow[20] made very extensive investigations on the possible substances which will reduce phosphomolybdic acid. They selected aminonaphthol sulphonic acid 1.2.4. for phosphorus reduction in the cold, and found that this substance gave a more intense blue color than all others tried by them.

Photometric Applications of Colorimetric Technic.—On theoretical grounds we considered the following three methods out of the list given previously, most suitable for investigation: (1) Fiske and Subarrow,[20] (2) Benedict and Theis,[13] (3) Youngburg and Youngburg.[19]

Attempted Photometric Application of Fiske and Subarrow Method.—Photometric application of this principle was found impracticable owing to the extreme instability of the aminonapthol sulphonate reagent. The reducing strength of this solution apparently varies from hour to hour. While this fact does not impair its utility for colorimetric purposes, it would necessitate preparing a fresh graph for each photometric estimation. This method was therefore abandoned as unsuitable.

Experimental Results Using Photometric Application of Benedict and Theis's Colorimetric Technic.—The following technic was adhered to: To 1 c.c. clear plasma add 2 c.c. of 20 per cent trichloracetic acid and 2 c.c. of distilled water. Shake and stand for five minutes. Filter through a phosphorus-free filter paper. To 2 c.c. of the filtrate add 5 c.c. of water, 1 c.c. of hydroquinone bisulphite solution, and 1 c.c. of molybdic acid solution. Place in a boiling water-bath for ten minutes. Cool under running water and read in the photometer.

In applying this technic we find that particular care must be taken to ensure constant conditions. Benedict and Theis recommend fifteen minutes boiling. The figures given in Table IV indicate that this period is too long. The greenish tinge which appears after ten minutes depresses optical density. We ensured standard conditions by the following means.

TABLE IV

P. CONT. OF SOL.	TIME OF BOILING	OPTICAL DENSITY	
0.020 mg.	5 min.	35	
	7 min.	45	
	9 min.	65	
	11 min.	64	
	13 min.	60	Greenish tinge
	15 min.	57	
	17 min.	57	
	19 min.	50	

The tubes were placed in a boiling water-bath adjusted so that the contents reached boiling within one minute.

At the end of ten minutes the tube was taken out and cooled to room temperature under running water. If the tube is allowed to cool spontaneously a considerable loss of time is involved, and the chances of error are greater. Oxidation is apt to occur at the top of the tube even if stoppered.

A graph relating optical density to concentration was prepared from a standard solution of KH_2PO_4 as shown in Table V.

TABLE V

AMT. OF P. IN SOL.	EQUIV. % OF P. USING 2 C.C. OF FILTRATE	OPTICAL DENSITIES		
0.005 mg.	1.25 mg.	16	17	17
0.010 mg.	2.50 mg.	34	34	34
0.015 mg.	3.75 mg.	52	52	52
0.020 mg.	5.00 mg.	68	69	68
0.025 mg.	6.25 mg.	89	85	86
0.030 mg.	7.50 mg.	105	106	106
0.035 mg.	8.75 mg.	126	125	125
0.040 mg.	10.00 mg.	143	144	143

Using a sample of human blood plasma, the method was tried out against the original colorimetric technic, and the following figures were arrived at:

PHOTOMETRIC METHOD	COLORIMETRIC METHOD
4.20 mg. % P.	4.0 mg. % P.
4.25 mg. % P.	4.25 mg. % P.
4.30 mg. % P.	4.10 mg. % P.
4.25 mg. % P.	4.20 mg. % P.

A long series of parallel estimations showed that it was possible to get good results with the above technic. On the other hand the method necessitated extreme care with the boiling conditions and other manipulative details. The method was also a lengthy one. All these factors decided us against adopting it for routine purposes.

Experimental Results Using Photometric Application of Youngburg and Youngburg's Method.—We next attempted a photometric application of Youngburg and Youngburg's method. The theoretical advantages were:

1. Small amount of blood required.
2. Stability and purity of reagents, none of which are organic.
3. Specificity for phosphorus.
4. Saving of time in manipulation.

First we proceeded to apply the original technic as given by Youngburg and Youngburg, with the following exception. For inorganic phosphorus we used 1 c.c. of filtrate representing 0.2 c.c. of plasma, instead of 2 c.c. as in the original technic. For total acid soluble phosphorus we took 1 c.c. of blood filtrate, instead of 2 c.c. as in the original technic. A graph was prepared using a standard solution relating optical density to concentration as shown in Table VI.

TABLE VI

ACTUAL P. IN SOL.	EQUIV. % P.	OPTICAL DENSITY
0.002 mg.	1 mg.	16
0.004 mg.	2 mg.	31
0.006 mg.	3 mg.	45
0.008 mg.	4 mg.	60
0.010 mg.	5 mg.	74
0.012 mg.	6 mg.	88
0.014 mg.	7 mg.	103
0.016 mg.	8 mg.	117
0.018 mg.	9 mg.	131
0.020 mg.	10 mg.	144

Before proceeding to carry out estimations of phosphorus in blood, we made a series of experiments to test the accuracy of the method.

Effect of Substances in Physiologic Quantities on Color Produced.—Table VII shows the results arrived at when substances usually met with in blood plasma were added in physiologic or somewhat larger quantities.

TABLE VII

SUBSTANCE ADDED	AMOUNT OF PHOSPHORUS FOUND				
	MG.	MG.	MG.	MG.	MG.
Nil	0.020	0.016	0.012	0.008	0.004
0.5 c.c. 20% Trichloracetic acid	0.0199	0.0158	0.0120	0.0082	0.0041
0.2 c.c. 2% Urea	0.0196	0.0160	0.0118	0.0078	0.0040
0.2 c.c. 1% NaCl	0.020	0.0158	0.0120	0.0082	0.0040
0.2 c.c. 1% $MgCl_2$	0.0202	0.0156	0.0120	0.0084	0.0040
0.2 c.c. 1% $CaCl_2$	0.0206	0.0158	0.0120	0.0080	0.0038
0.2 c.c. 0.1% Uric Acid	0.0204	0.0160	0.0120	0.0078	0.0038
0.2 c.c. 2% Glucose	0.020	0.0160	0.0120	0.0084	0.0038
0.2 c.c. 0.5% K_2SO_4	0.0206	0.0166	0.0120	0.0076	0.0034

It will be seen that the color produced is not affected by substances even well over physiologic limits. The small differences are no greater than could be attributed to experimental error, with one exception. It will be noticed that sulphates do have a definite although small effect. This point will be taken up again when we consider the method as applied to the analysis of total acid soluble and lipoid fractions in blood.

Effect of Varying Amounts of Stannous Chloride.—Kuttner and Cohen[18] state that the limits of concentration of stannous chloride for color development are 0.020 and 0.022 per cent stannous chloride in the final solution. Kuttner and Lichtenstein[21] on the basis of further work state that this is too conservative and that the optimal zone of color production can be extended to 0.01-0.03 per cent. The figures in Table VIII tend to confirm that the original limits as given by Kuttner and Cohen are too narrow. It will be seen that very little variation in color is produced between such wide limits as 0.15-0.3 per cent.

TABLE VIII

P. CONT.	OPTICAL DENSITY WITH VARYING CONCNS. OF STANNOUS CHLORIDE					
	0.005% $SnCl_2$	0.01% $SnCl_2$	0.015% $SnCl_2$	0.02% $SnCl_2$	0.03% $SnCl_2$	0.04% $SnCl_2$
0.004 mg.	34	32	31	31	29	34
0.008 mg.	65	62	59	60	58	67
0.012 mg.	103	95	90	88	85	90
0.016 mg.	142	129	120	117	118	124
0.020 mg.	170	152	146	144	142	148
Nil	14	15	20	20	26	27

Satisfied with these preliminary experiments we proceeded to test out the method on "routine bloods" in the laboratory.

Experimental Results Using Blood Plasma.—A. Estimation of Inorganic Phosphorus Fraction: Youngburg and Youngburg's original technic was adhered to. The reader will find details of the technic as modified for photometric use, at the end of this article. Estimations in triplicate on two bloods gave the following typical results:

a. 3.4 3.35 3.35 mg. per cent Phosphorus
b. 3.80 3.80 3.80 mg. per cent Phosphorus

In order to test the accuracy of these figures we carried out the following experiments.

1. Parallel estimations in comparison with the photometric application of Benedict and Theis's method (Table IX).

TABLE IX

NAME	BENEDICT'S ADAPT.	YOUNGBURG & YOUNGBURG ADAPT.
M.M.	4.8 mg. % P.	4.7 mg % P.
F.A.	5.2 mg. % P.	5.15 mg. % P.
F.V.	4.7 mg. % P.	4.5 mg. % P.
MM (serum)	4.6 mg. % P.	4.5 mg. % P.
	4.6 mg. % P.	4.6 mg. % P.
	4.8 mg. % P.	4.6 mg. % P.
F.	4.5 mg. % P.	4.7 mg. % P.
	4.6 mg. % P.	4.8 mg. % P.
O'Br.	5.2 mg. % P.	4.6 mg. % P.
	5.0 mg. % P.	5.1 mg. % P.
H.	3.35 mg. % P.	3.6 mg. % P.
XCIV	4.6 mg. % P.	4.7 mg. % P.
	4.6 mg. % P.	4.7 mg. % P.
	4.6 mg. % P.	4.6 mg. % P.
	4.8 mg. % P.	4.6 mg. % P.

2. Parallel estimations with a macromethod using large amounts of animal blood (Table X).

TABLE X

MICRO ESTIMATION (0.2 C.C. PLASMA USED)	MACRO ESTIMATION (120 C.C. PLASMA USED)
3.5 mg. per cent	3.30 mg. per cent
3.5 mg. per cent	3.30 mg. per cent
3.6 mg. per cent	3.50 mg. per cent
3.5 mg. per cent	3.46 mg. per cent
3.5 mg. per cent	3.46 mg. per cent

(The macromethod was a titrimetric application of Neuman's method of precipitation of ammonia phosphomolybdate, the actual technic being an application of the method given by Rona and Kleinmann[21] for phosphorus in urine.)

3. Recovery of known quantities of phosphorus added to blood plasma (Table XI).

TABLE XI

ORIG. P. CONT. OF BLOOD	PHOS. ADDED	PHOS. FOUND	PHOS. RECOVERED	% PHOS. RECOVERED
0.0071 mg. P.	0.002 mg.	0.0092 mg.	0.0021 mg.	105
	0.002 mg.	0.0092 mg.	0.0021 mg.	105
	0.004 mg.	0.0111 mg.	0.0040 mg.	100
	0.004 mg.	0.0113 mg.	0.0042 mg.	105
	0.006 mg.	0.0132 mg.	0.0061 mg.	102
	0.006 mg.	0.0132 mg.	0.0061 mg.	102
0.0041 mg. P.	0.002 mg.	0.0061 mg.	0.0020 mg.	100
	0.004 mg.	0.0081 mg.	0.0040 mg.	100
	0.006 mg.	0.0100 mg.	0.0059 mg.	98.4
	0.008 mg.	0.0121 mg.	0.0080 mg.	100
	0.012 mg.	0.0160 mg.	0.0119 mg.	99.7

B. Estimation of Acid Soluble and Lipoid Fractions: When we proceeded to apply the technic, with the slight quantitative modifications mentioned above, to the analysis of the acid soluble and lipoid phosphorus fractions, we found difficulties during the process of oxidation, which we shall go into in some detail.

Youngburg and Youngburg discussed the use of H_2O_2 as an oxidizer, first introduced for phosphorus estimation by Baumann.[22] We agree in finding it far superior to the other oxidizers for this purpose. Even here the excess of H_2O_2 must be removed. A green color is produced with the molybdic acid solution if the slightest trace is present, and the development of a blue color on addition of stannous chloride is inhibited. We were therefore careful to chase out the final traces of H_2O_2 by following the flame from the bottom to the top of the tube several times. When during the course of these experiments we were obliged to make up new quantities of molybdic acid, we noticed that results were not concordant with previous findings. On titration we found that the acid content of the "10/N" acid from a different Winchester was slightly deviated. With reference to this subject, Kuttner and Cohen[18] state that the limits of sulphuric acid in the final solution should be between 0.9/N and 1.05/N. In our experience this range is confirmed for specificity of the method for phosphorus. We were however, unable to agree that the color development with an equal amount of phosphorus in the solution, is equal for 0.90 and 1.05/N respectively. We found that extraordinarily small deviations in the normality of the acid had a definite effect on the optical density of the color produced. This factor also agreed with the definite effect on color production of adding sulphates to an ordinary solution, as mentioned in Table VII. Thus we found it necessary to plot out a fresh graph for the standard phosphorus solution whenever a new batch of reagents were prepared. From the point of view of photometric technic, a graph is of use only when the amount of sulphuric acid in the unknown solution is rigidly identical with that which was

present in the graph solution. Following on from this we were led to go into the whole question of oxidation. When the original technic of Youngburg and Youngburg is adhered to, it is a fact that some H_2SO_4 is used in the oxidation process, and that some is also lost in the sweeping out of the final traces of H_2O_2 from the combustion tube.

Using great care to avoid unnecessary loss of H_2SO_4 we carried out experiments to assess the degree of error introduced by loss of acid.

Using 3 c.c. of plasma divided into three equal parts, we prepared three trichloracetic acid filtrates in the usual way. One and five-tenths cubic centimeters of each filtrate was used, and heated with 0.75 10/N sulphuric acid and oxidized with H_2O_2. The solution was then boiled, cooled and completed to 15 c.c. This 15 c.c. was divided into three equal parts for the purposes of the experiment. (In fact only 4 c.c. could be used for one part, owing to unavoidable pipette error.) The three portions of 15 c.c. each were utilized as follows:

1. Twenty-five hundredths of a cubic centimeter of 10/N H_2SO_4 was added to the 5 c.c. and the analysis completed exactly as per Youngburg and Youngburg's modified technic, as adapted to photometric use. The amount of phosphorus found in each of the three specimens is

   0.00422 mg.
   0.00407 mg.
   0.00407 gm.

2. Five cubic centimeters was titrated to find the sulphuric acid content. We found:
   [0.25 c.c. 10/N H_2SO_4 gave an equivalent figure of 2.54 c.c. of N NaOH]
   1. 5 c.c. of solution contained 2.36 c.c. N H_2SO_4
      $\therefore$ Loss = 0.18 N H_2SO_4
   2. 5 c.c. of solution contained 2.38 c.c. N H_2SO_4
      $\therefore$ Loss = 0.16 N H_2SO_4
   3. 5 c.c. of solution contained 2.4 c.c. N H_2SO_4
      $\therefore$ Loss = 0.14 N H_2SO_4

3. Four cubic centimeters was corrected for lost sulphuric acid before completing the estimation, and our findings were as follows:

[As the volume of solution was 4 c.c. instead of 5 c.c., it was necessary to add 0.3 c.c. 10/N H_2SO_4 to the 4 c.c.]

SUPPLEMENTARY H_2SO_4 ADDED	PHOS. CONT.		CORRECTED FOR 5 C.C.
1. 1.44 c.c.	0.00316	=	0.00395 mg.
2. 1.28 c.c.	0.00316	=	0.00395 mg.
3. 1.12 c.c.	0.00316	=	0.00395 mg.

A similar series of experiments were carried out on the Lipoid fractions: Three portions of the same plasma were precipitated and extracted in 10 c.c. alcohol ether. Six cubic centimeter of each filtrate was oxidized with 1 c.c. 10/N H_2SO_4. After boiling the oxidate was completed to 15 c.c.

This solution was utilized as follows:

1. Five cubic centimeters of the solution was estimated exactly as per the Youngburg and Youngburg modified technic as adapted to photometric use. We found:
   1. Phosph. contained = 0.01020 mg.
   2. Phosph. contained = 0.00990 mg.
   3. Phosph. contained = 0.01005 mg.

2. Four cubic centimeters of solution was titrated to estimate acid lost in oxidation. [0.5 c.c. 10/N acid gave 5.08 c.c. N/1 NaOH]
   1. 3.47 c.c. of N NaOH reqd. i. e. 4.34 c.c. for 5 c.c.
      Loss = 0.74 c.c. N.
   2. 3.56 c.c. of N NaOH reqd. i. e. 4.45 c.c. for 5 c.c.
      Loss = 0.63 c.c. N.
   3. 3.54 c.c. of N NaOH reqd. i. e. 4.42 c.c. for 5 c.c.
      Loss = 0.66 c.c. N.

3. Five cubic centimeters of the solution were corrected for lost acid and the phosphorus content ascertained. We found:

	SUPPLEMENTARY ACID ADDED	PHOS. CONT.
1.	0.74 c.c.	0.00944
2.	0.63 c.c.	0.00944
3.	0.66 c.c.	0.00924

These results are summarized in Table XII.

TABLE XII

ACID SOLUBLE FRACTION			LIPOID FRACTION		
DIRECT ESTM.	MODIFICATION ADDING LOST ACID	% ERROR	DIRECT ESTM.	MODIFICATION ADDING LOST ACID	% ERROR
0.00422 mg.	0.00395 mg. P.	6.9%	0.01020 mg.	0.00944 mg. P.	7.5%
0.00406 mg.	0.00395 mg. P.	2.6%	0.00990 mg.	0.00944 mg. P.	4.7%
0.00406 mg.	0.00395 mg. P.	2.6%	0.1005 mg.	0.00924 mg. P.	7.6%

These findings show a percentage error at least sufficiently great to be taken into consideration. This would in our experience also apply to the original Youngburg and Youngburg colorimetric technic. We suggest therefore, that workers using this technic should take cognizance of this source of error and correct for it.

To summarize our conclusions derived from the experimental results with Youngburg and Youngburg's technic described above, we finally came to the conclusion that the original Youngburg and Youngburg technic is suitable for photometric adaptation to the analysis of inorganic phosphorus. It is not suitable for the estimation of total acid soluble and lipoid fractions without modification. If, however, any acid lost in the oxidation process is collected, it gives excellent results.

DETAILED DESCRIPTION OF PHOTOMETRIC TECHNIC FINALLY ADOPTED

For the use of workers who intend to use the photometer for routine analysis of phosphorus fractions in blood, we append the exact technic used in this laboratory. For the establishment of the preliminary graph using a standard KH_2PO_4 solution, we refer readers to Table V. We desire also to call attention to the necessity of using clear plasma (or serum), which must be free from any traces of hemolysis. The routine followed in our laboratory is to use a portion of the plasma collected under paraffin for the estimation of blood alkaline reserve; this is rapidly separated off from the corpuscles by centrifugalization.

Reagents.—1. Sodium Molybdate solution
112 gm. molybdic acid in 450 c.c. water. Neutralize with caustic soda (about 54 gm.). Boil for thirty minutes and make up to 200 c.c.

2. Sulphuric Acid
37.5 c.c. sulphuric acid added to 100 c.c. water.

3. Molybdic Acid Solution No. 1
50 c.c. 7.5 sodium molybdate plus 50 c.c. 10/N sulphuric acid.

4. Molybdic Acid Solution No. 2
50 c.c. 7.5 sodium molybdate plus 50 c.c. 5/N sulphuric acid.

5. Stannous Chloride
10 gm. stannous chloride in 25 c.c. conc. hydrochloric acid. Dilute 1 c.c. to 200 c.c. with water for use.

6. Alcohol-Ether
75 c.c. alcohol mixed with 25 c.c. ether.

7. Twenty per cent Trichloracetic acid

8. Hydrogen peroxide (40 vols.)

9. N/1 H_2SO_4 solution (must be exact)

10. N/1 NaOH solution (must be exact)

Technic.—Preparation of Filtrate: To 0.8 c.c. plasma add 1.6 c.c. water, mix in a test tube. Then add 1.6 c.c. 20 per cent trichloracetic acid and shake vigorously. Allow the mixture to stand for fifteen minutes, filter.

1. *Inorganic Phosphorus Fraction:* Take 1 c.c. of the filtrate, 6 c.c. water, and 2 c.c. molybdic acid solution No. 1. Mix, add quickly 1 c.c. of the dilute stannous chloride solution. Allow to stand for about five minutes, read the depth of color produced in the photometer.

Prepare a control tube, and replace the filtrate by distilled water. Note the difference in these two readings on the special graph giving the result in mg. phosphorus per 100 c.c. blood.

2. *Total Acid Soluble Phosphorus Fraction:* Take 1.2 c.c. of the protein-free filtrate. Add 0.6 c.c. of 10/N sulphuric acid in a Pyrex tube. Heat with addition of hydrogen peroxide until a clear solution is obtained, taking care (a) to avoid unnecessary loss of sulphuric acid, and (b) to insure sweeping out excess of hydrogen peroxide in the tube. Cool, add 2 c.c. water and boil. Cool, transfer quantitatively to a 10 c.c. graduated cylinder. Complete to 6 c.c. and mix.

Pipette out 1 c.c. of the solution into a small flask. Titrate with N/1 soda from a microburette. Calculate the amount of sulphuric acid in the remaining 5 c.c. Do a control titration at the same time on 0.5 c.c. of 10/N sulphuric acid, thus calculating the amount of sulphuric acid lost during combustion. Add the amount of sulphuric acid lost using N H_2SO_4 to the 5 c.c. in the cylinder, complete to 7 c.c. with water, add 2 c.c. molybdic acid solution No. 2 and 1 c.c. stannous chloride. Read in the photometer after five minutes.

3. *Lipoid Phosphorus Fraction:* Pipette 3 c.c. of the ether-alcohol mixture into a test tube with a 4 c.c. graduation mark, using a fine bore pipette add 0.2 c.c. plasma slowly, cork and shake well, bring to boil, stand for ten minutes, cool, complete to 4 c.c., shake to mix, and filter. Then 2.4 c.c. of filtrate is evaporated to dryness in a hard glass test tube. Six-tenths of a cubic centimeter of 10/N sulphuric acid is added, and the contents cautiously heated and oxidized with hydrogen peroxide. The subsequent estimation of phosphorus is as for acid soluble phosphorus, care being taken to make good any acid lost in combustion.

SUMMARY

1. It is claimed that the Vernes-Bricq-Yvon photometer represents an advance over all other colorimeters and nephelometers commonly used.

2. Reasons are given for this claim. A table is given showing the chemical constituents of blood, urine and feces, for the analysis of which photometric methods have been evolved in this laboratory. As an illustration of the steps taken to evolve rapid photometric methods for routine laboratory use, the estimation of phosphorus fractions in blood is discussed in detail.

3. Existing nephelometric methods for blood phosphorus estimation are discussed. The photometric application of these methods is unpractical. Reasons are given for this statement.

4. Existing microcolorimetric methods for blood phosphorus estimation are discussed.

5. Experiments are discussed showing the attempted application of the animonapthol sulphonic acid 1.2.4. phosphorus reduction method. Reasons are given for its rejection.

6. A description is given of an attempted photometric application of Benedict and Theis's modification of Briggs colorimetric method. It is found to give accurate figures. Its disadvantages are given as reasons for not adopting it as a routine laboratory method.

7. A photometric application of Youngburg and Youngburg's colorimetric method is described.

8. Experiments are described proving the accuracy of this method for the estimation of the inorganic phosphorus fraction in blood.

9. Experiments are described showing the source of error in the oxidation process of Youngburg and Youngburg's technic. This renders Youngburg and Youngburg's technic inaccurate for the analysis of the total acid soluble and lipoid phosphorus fractions in blood.

10. A modification is suggested which does away with this source of error.

11. A microphotometric technic for the analysis of the phosphorus fractions in blood is given in detail. This method has been used for some time as a routine in the authors' laboratory, and found to be simple, time-saving and extremely accurate.

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PRELIMINARY REPORT ON THE USE OF AN IMPROVED FORM OF ELECTROCARDIOGRAPH*

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THE introduction by Einthoven of the string galvanometer, or electrocardiograph, in 1903, and its subsequent use in the laboratory and in the clinic marked an important advance in the understanding of certain cardiac conditions.

There are now available for electrocardiography many models of the string galvanometer type and a few combining a vacuum tube amplifier with an oscillograph. Doubt has been cast upon the accuracy of the latter type of instrument.[1] Ernstene and Levine[2] obtained records with both instruments and found only small differences in the records; they concluded that the amplifier-oscillograph type of electrocardiograph gave records of sufficient accuracy to warrant continuation of its use, basing this conclusion upon the assumption that the string galvanometer was a suitable standard of comparison. This assumption, however, in view of the results discussed below, is not tenable. Actually, the results obtained from the two types should be different if the amplifier-oscillograph equipment is properly designed.

DEFECTS OF THE STRING GALVANOMETER

It would seem that the medical profession has assumed that the string galvanometer is a suitable instrument, in the sense that it will faithfully record the variations in electrical potential that occur in the human heart. However, this opinion is not concurred in by electrical engineers; they use the oscillograph almost exclusively for recording such variations in electric potential.

A detailed discussion of the defects of the string galvanometer is about to be published by members of the Department of Electrical Engineering of the Massachusetts Institute of Technology; I am permitted to abstract some of this article.[3] Two significant defects are discussed. First it is shown that the wave forms recorded by the string galvanometer become distorted when frequencies much in excess of 20 cycles per second are present. In addition a wave (the QRS from a normal electrocardiogram) was subjected to what is known as harmonic analysis, a form of mathematical analysis, and it was disclosed that the frequency of the components involved greatly exceeded 20 cycles per second. It is stated[3]: "The relative amplitudes show that these higher harmonics are of great importance in determining the wave form, and any failure to respond equally at both high and low frequencies must result in distortion of the wave."

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The second source of error in the recording of changes of electrical potential, i.e., in the electrocardiogram, by the string galvanometer lies in the presence of body resistance in the measuring circuit. To quote[3]: "Under average conditions, the resistance of the human body is about 2500 ohms between terminals such as are used in electrocardiographic studies. The galvanometer resistance is usually of the same order of magnitude. Hence the current which flows in the galvanometer is controlled as much by the internal resistance of the body as by the external resistance of the measuring circuit. Although the galvanometer resistance is substantially constant, the body resistance may vary and thus alter the shape of the wave on the record."

USE OF AN IMPROVED FORM OF ELECTROCARDIOGRAPH

An apparatus is described[3] which more suitably fulfills the principle requirements of any electrocardiograph, which are: first, it must produce records which accurately depict the variations of electromotive force impressed upon it; second, the records thus produced should be of sufficient size to permit accurate interpretation. It consists principally of a special tube amplifier and an oscillograph.

In the following is presented a preliminary report of the application of this new instrument in the field of electrocardiography, and a few records obtained by its use.

Approximately 70 tracings have been taken of 56 patients, on both the string galvanometer and the new equipment. An analysis of the voltage recorded, or

TABLE I

COMPARISON OF VOLTAGE RECORDED BY AMPLIFIER-TYPE AND BY IMPROVED FORM OF ELECTROCARDIOGRAPH WITH THAT RECORDED BY AN EINTHOVEN STRING GALVANOMETER; TWO SERIES OF 25 PATIENTS IN EACH COMPARISON

WAVE	STATUS[1]	NUMBER OF LEADS[4]		AVERAGE DIFFERENCE[5]		MAXIMAL DIFFERENCE[5]	
		AMP.[2]	IMP.[3]	AMP.[2]	IMP.[3]	AMP.[2]	IMP.[3]
P	same	23	19				
	lower	48	15	0.02	0.02	0.05	0.07
	higher	4	41	0.01	0.04	0.01	0.18
Q	same	49	37				
	lower	21	12	0.03	0.02	0.05	0.06
	higher	5	26	0.02	0.03	0.02	0.13
R	same	10	3				
	lower	64	24	0.10	0.09	0.37	0.34
	higher	1	48	0.08	0.19	0.08	0.90
S	same	28	34				
	lower	43	31	0.05	0.09	0.20	0.34
	higher	4	10	0.05	0.04	0.10	0.11
T	same	32	23				
	lower	41	12	0.05	0.03	0.11	0.05
	higher	2	40	0.01	0.03	0.01	0.15

1. As compared with same wave recorded by string galvanometer.
2. The amplifier-type instrument tested by Ernstene and Levine.[2]
3. The improved form of electrocardiograph reported in this article.
4. Standard three leads for each patient, so number of leads total 75.
5. In millivolts.

the amount of the excursion from the baseline, shows that it is not identical in the records obtained by the two instruments. The details are given in Table I, in which is included a summary of the results obtained by Ernstene and Levine[2] in their comparison of the electrocardiograms taken by the Einthoven string galvanometer and an amplifier-type electrocardiograph. Their comparison was based upon a series of twenty-five cases, as is ours in Table I. The fact that the

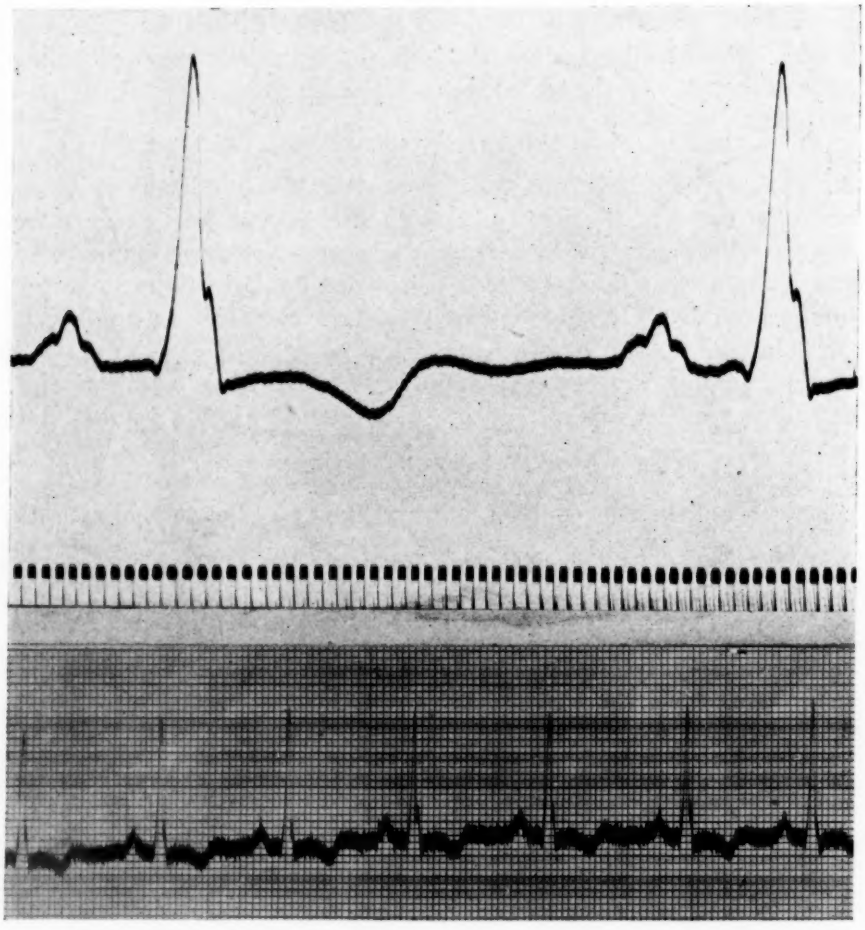

Fig. 1.—Normal rhythm from case of rheumatic mitral stenosis. Note difference in P-waves and R-waves. In this and in the subsequent illustrations the new electrocardiogram is mounted above, that taken by the string galvanometer type is below. The time marker, indicating one-fiftieth of a second, is at the bottom of new record. In the string galvanometer record the time intervals are indicated by the conventional abscissae, the fine ones equal one-twenty-fifth of a second, the coarse ones equal one-fifth of a second.

standard three leads were recorded in each of the 25 patients causes the figures given to total 75.

In brief, Table I presents a check, in amount of electrical potential recorded in millivolts, of the new apparatus against the string galvanometer and against the amplifier type of electrocardiograph tested by Ernstene and Levine.[2] Subject to the limitation that the patients used by the latter and by me were not

the same individuals, the suggestion is that their amplifier type of electrocardiograph and the new apparatus do not give identical results.

The latter equipment permits the spreading out of the electrocardiogram, if desired, to such a degree that but a single cardiac beat is recorded on a 36-inch strip of paper. The waves can be amplified, without loss of accuracy, to almost the full width of standard oscillograph paper ($3\frac{3}{4}$ inches). In the present study,

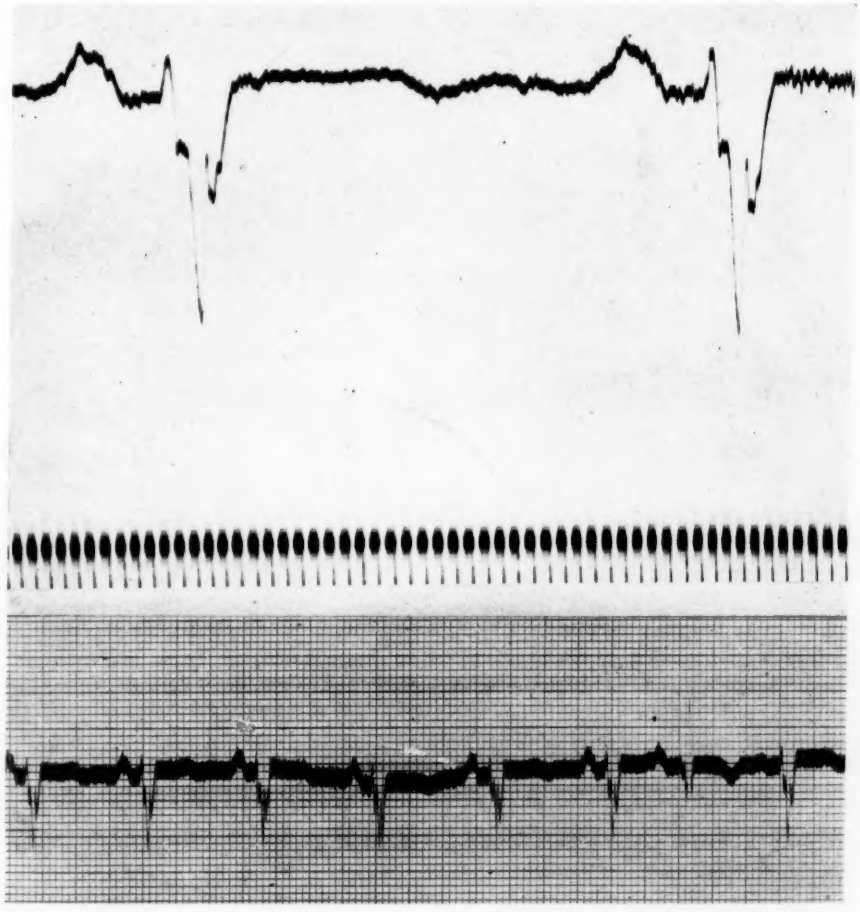

Fig. 2.—Normal rhythm. Note details of the QRS complex.

however, attention has been concentrated upon a practical (Figs. 1 to 6) rather than upon the freak size.

Some of the oscillations in Figs. 1 to 6 do not originate in the heart of the patient. Since these electrocardiograms were obtained, further investigation has been made by Caldwell, Oler, and Peters; they discuss[3] the sources of these fine oscillations and methods for their prevention.

DISCUSSION

There is a desire to utilize changes in detail of the electrocardiogram in the diagnosis of impairment of the myocardium, such as is prone to occur in the

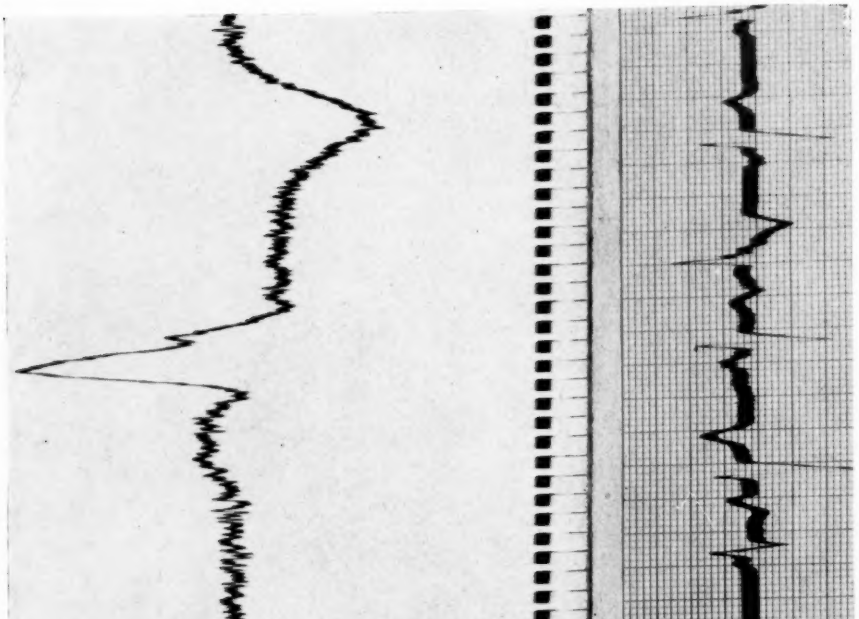

Fig. 3.—A normal beat followed by an extrasystole of ventricular origin. A considerable number of the fine oscillations, mentioned in the text, occur in this record.

presence of coronary thrombosis. There can be little dispute that, if small differences are to be of diagnostic value, the electrical record must be accurate. Confidence in the accuracy of the electrocardiogram cannot, in view of the work of Caldwell et al.,[3] be placed in records made by a string galvanometer; it would seem that it can if such or similar equipment, as they describe, be used.

Some waves are of insufficient amplitude and many notchings are too inconspicuous to be readily studied in electrocardiograms taken according to the present standardization on the instruments commonly used. This improved form of

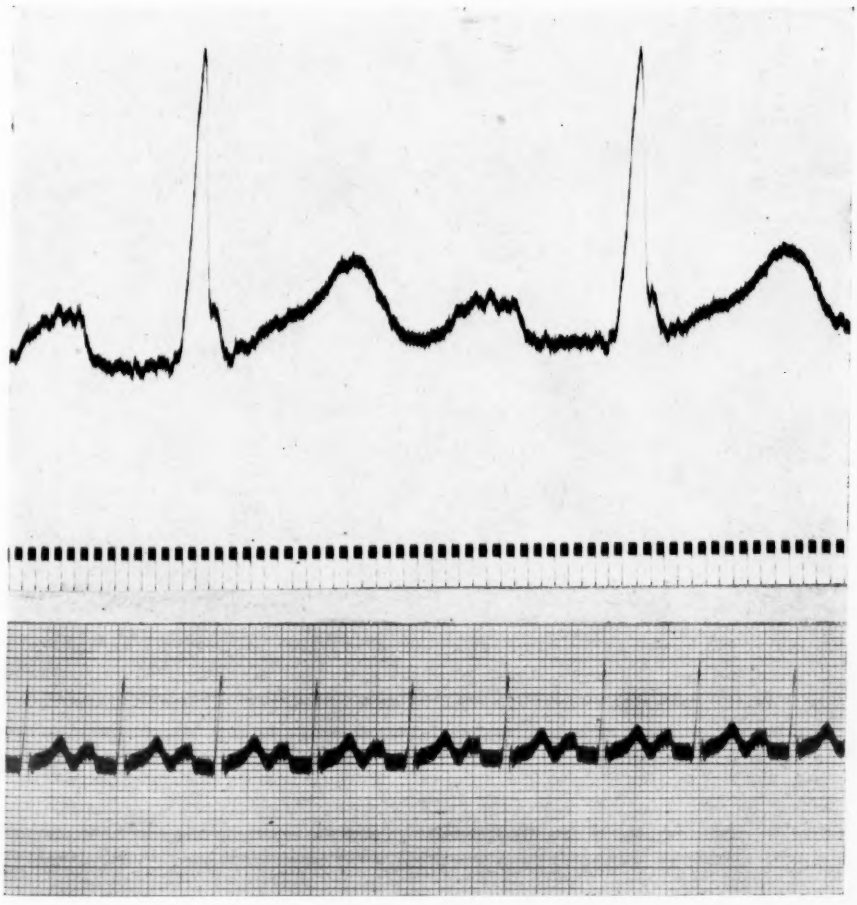

Fig. 4.—Delayed A-V conduction. The P-R interval measures 0.27 second.

electrocardiograph makes it possible to amplify and spread out the electrocardiograms without loss of accuracy. One accustomed to the study of electrocardiograms experiences the sensation that now he can study under high power, of the microscope, records which formerly could be viewed only under low power. Reference to Figs. 1 to 6 will disclose the manner in which the improved equipment records the barely visible notchings and thickenings of waves in the conventional type of electrocardiogram as definite notchings and alterations in contour of the respective waves.

It is distinctly probable that the standardization that is part of the conven-

tional technic of electrocardiography makes the record too small to properly disclose some of its details. Adoption of a technic that produces a somewhat larger and more spread out electrocardiogram, facilitated by this improved equipment, gives promise of disclosing new information pertaining to cardiac physiology. Examination of the electrocardiograms already obtained with this improved equipment gives me the temerity to advocate such a change in the standardization employed (provided the improved equipment, or similar, is used) in electrocardiography.

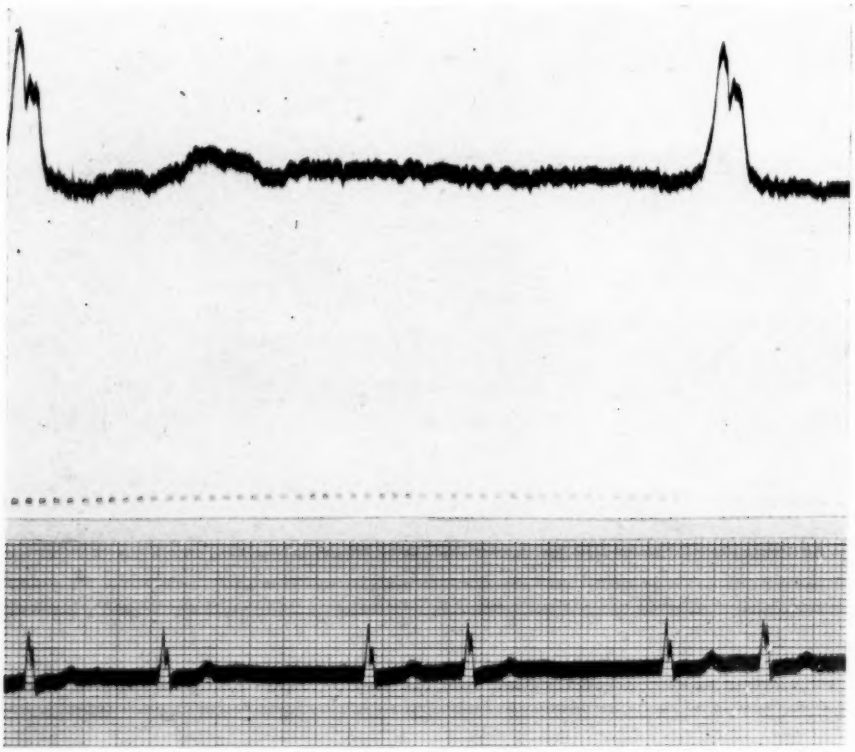

Fig. 5.—Auricular fibrillation. Some difference is apparent in the contour of the QRS complex.

It is appreciated that during recent years at least three electrocardiograph equipments using vacuum tube amplifiers with an oscillograph have appeared upon the market. These produce records similar in appearance to those made by the Einthoven string galvanometers. They undoubtedly possess some of the advantages of the equipment used in the instrument described by Caldwell, Oler, and Peters, but as the latter state[3]: "No attempt is made to improve upon the conventional type of record." It is emphasized that the workers at the Massachusetts Institute of Technology did not seek to reproduce the record obtained by the string galvanometer, but directed their efforts towards assembling an equipment that would produce the best type of record of the changes in electrical potential occurring in the human heart.

It is not possible as yet to state what will result from the use of this improved form of electrocardiograph in the field of electrocardiography. It may

take years, with some laboratory experiments and with many of these new electrocardiograms correlated with the clinical records and necropsy findings, to determine what new information, if any, can be obtained by the use of this more accurate and more efficient instrument. There can be little doubt that the opportunity afforded by the new equipment thoroughly warrants full and hopeful investigation.

There are numerous problems of research along physiologic lines which need an instrument more capable of recording minute changes in electrical potential.*

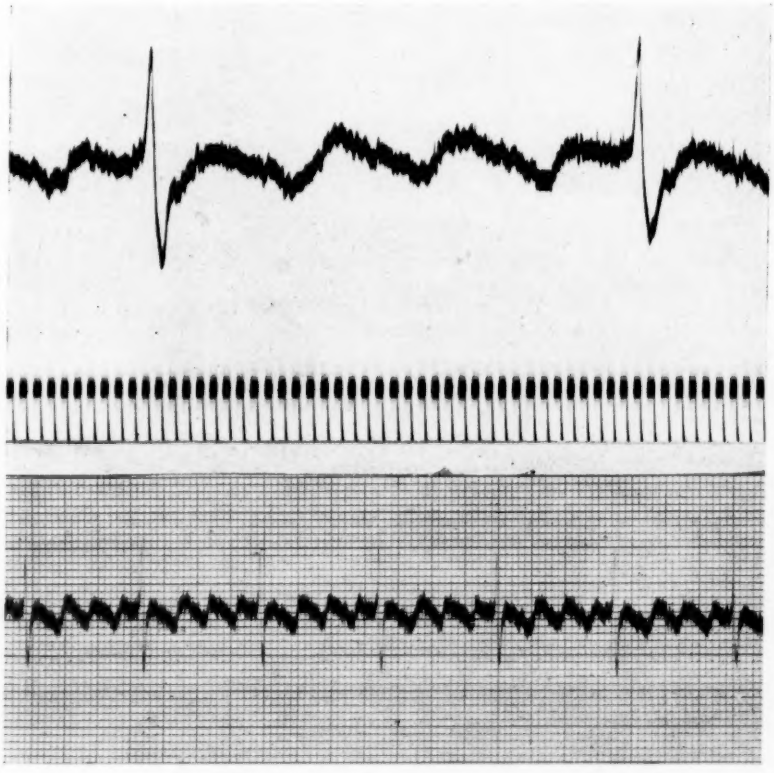

Fig. 6.—Auricular flutter.

It is believed that this improved form of electrocardiograph represents a suitable instrument.

This preliminary report is offered particularly for the information of those who desire to avail themselves of the opportunities afforded by a better instrument for electrocardiography and physiologic research.

SUMMARY

The Einthoven string galvanometer is not a suitable instrument for electrocardiography, in the sense that it does not faithfully record the rapid variations in electrical potential that take place in the heart.

*F. H. Pratt, Professor of Physiology at Boston University School of Medicine, already has recorded with this apparatus curves produced by the extremely low action potential of the anterior and posterior lymph hearts in the frog.

The improved form of electrocardiograph is suitable for such work. The workers at the Massachusetts Institute of Technology directed their investigation to the assembling of an equipment that would faithfully record the rapid changes of electrical potential occurring in the heart, and that would produce the best form of record, rather than of attempting to reproduce the conventional electrocardiogram.

This improved equipment permits the amplification and spreading out of the electrocardiogram, without loss of accuracy, so that the details of its waves may be studied. Adoption of a technic that produces a somewhat larger and more spread out record is advocated.

It seems hopeful that the future use of this improved form of electrocardiograph will disclose new information pertaining to the heart.

This improved equipment should also be of definite assistance in the pursuit of problems in physiology requiring the recording of rapid changes in electrical potential.

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ANEROID-TYPE OF TAMBOUR FOR RECORDING RESPIRATORY MOVEMENTS AND INTRATHORACIC PRESSURE*

BURGESS GORDON, M.D., PHILADELPHIA

CERTAIN difficulties are experienced with the rubber diaphragm type of tambour in recording changes in respiration. The tension of the diaphragm varies and the thread which secures the rubber to the bell of the instrument may become loose. As a result fallacies may occur in the tracing and not infrequently it is necessary to adjust the apparatus during the experiment. Since the tension of the rubber may vary, comparative studies with the same "set up" are not entirely correct. Furthermore the rubber may disintegrate due to heat, the action of foreign substances and when not in use. To replace the rubber is time-consuming.

The following is a description of an instrument which has been found useful in recording changes in the intrathoracic pressure, the rate and depth of respirations in man and animals. A housing 7.5 cm. in length and 3.5 cm. in width (divided into two compartments) contains aneroid and eccentric units. The aneroid consists of five chambers which are constructed from "crimped" German silver sheeting .025 mm. in thickness. A valve stem with an opening into each chamber passes through the center of the aneroid and into the outer

*From the medical service of Dr. Thomas McCrae and the Department for Diseases of the Chest, Jefferson Hospital.
Received for publication, December 8, 1931.

wall of the housing where it is connected with brass tubing 14 cm. in length. This tubing serves as the conduit for air and is the part for attachment of the tambour to the upright stand. The distal end is connected with the rubber tubing which leads to the pneumothorax needle or chest recording hose. The other end of the valve fits into a collar which is screwed to a shaft 0.5 cm. in length. This passes through the partition and into the other compartment where it is connected by means of a pinion to the lateral arm of the eccentric. The central part of the eccentric is clamped to a concave movable base which

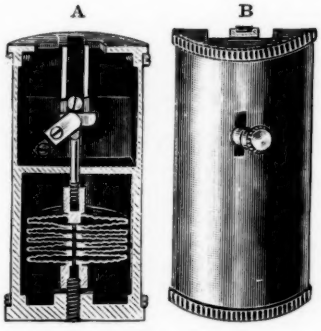

Fig. 1

Fig. 2

Fig. 1.—*A*. This is a cross-section showing the aneroid and eccentric units. *B* shows the set screw which regulates the "swing" of the eccentric and recorder.

Fig. 2.—Shows the tambour attached to the upright stand and a section of the rubber tubing for connection with the pneumothorax needle or chest recording hose.

fits closely in the lateral section of the compartment. The aluminum recorder is clamped in a box which projects from the central part of the eccentric. A partial rotation of the eccentric converts the horizontal motions of the aneroid into vertical movements. The arm and recorder have free excursion through an opening 2.5 cm. in width at the end of the chamber. The base of the eccentric is adjustable by means of a set screw. This provides a means for regulating the degree of recorder excursion above or below the neutral line.

The apparatus is light in weight, easily portable, and comparatively rugged in construction.

The instrument is manufactured by Geo. P. Pilling and Son Company, Philadelphia.

A SIMPLIFIED INSTRUMENT FOR MEASURING METABOLISM*

By Allen D. Garrison, Ph.D., Houston, Texas

IT HAS occurred to the author that, with the growing demand for measurements of basal metabolism, the instruments available for the purpose present some decided disadvantages from the standpoint of many physicians and technicians who would like to be prepared to make such measurements.

The first disadvantage is one of cost. It would appear that many physicians, particularly those in small towns or otherwise isolated from well-equipped laboratories and hospitals, would frequently have occasion to measure basal metabolism, but hesitate to invest $200 to $700 in one of the instruments now available.

Furthermore, one familiar with metabolism measurements will also recognize inconveniencies other than first cost: instruments which record oxygen absorption rates by a kymograph record, such as that used in the Benedict-Roth or the McKesson designs, have the disadvantage of certain inaccuracies due to irregular breathing, and the resultant serious difficulty of estimating the average slope of a line drawn along the base of a second very wobbly curve; the demand for pure oxygen often amounts to an inconvenience, since tanks must often be shipped from a distance; and while the Douglas, Bailey and similar methods avoid both of these troubles, one is required to be a fairly good gas chemist with considerable capital.

The apparatus illustrated in Fig. 1 has been designed to eliminate, as far as possible, some part of the inconveniencies and inaccuracies of metabolism measurements and, at the same time, to fill a need which may not be completely filled by more complicated and expensive instruments; namely, to supply a simple, durable, portable, yet inexpensive, and accurate device.

The principle of the measurement is a departure from previous practice. This instrument collects exhaled air over a measured time (a definite number of exhalations), and measures the entire amount of carbon dioxide exhaled in that time. By the use of the respiratory quotient, it is a simple matter to calculate as if oxygen had been measured directly.[1]

(*A*) is a rigid container for the exhaled air which is led into the space (*B*) from the subject through an ordinary mouthpiece and flexible tubing, containing flutter valves (not shown in the illustration). Exhaled air coming through the flexible tubing (*N*) and the tube (*M*) may be directed either into the space (*B*) of the container (*A*), or it may be deflected out into the outside air by turning the four-way valve (*K*).

A diaphragm (*D*), operating on the principle of a gasometer, divides the container into compartments (*B*) and (*C*), and can be operated by pushing or pulling the rod (*E*) which is equipped with a handle at its upper end and

*From the Department of Physical Chemistry, The Rice Institute.
Received for publication, November 3, 1931.

emerges through the top of the container through a packing joint (*F*) with a knurled nut for adjusting the compression on the packing. It will be evident that the gasometer diaphragm (*D*) controls the relative volumes of the spaces (*B*) and (*C*), and that the water in the lower half of the container serves as a gas-tight seal between these spaces, whether the diaphragm is in the raised or lowered position. The volume of water necessary to effect this seal is reduced by constructing the bottom of the container in the manner illustrated, thereby reducing the weight of the apparatus.

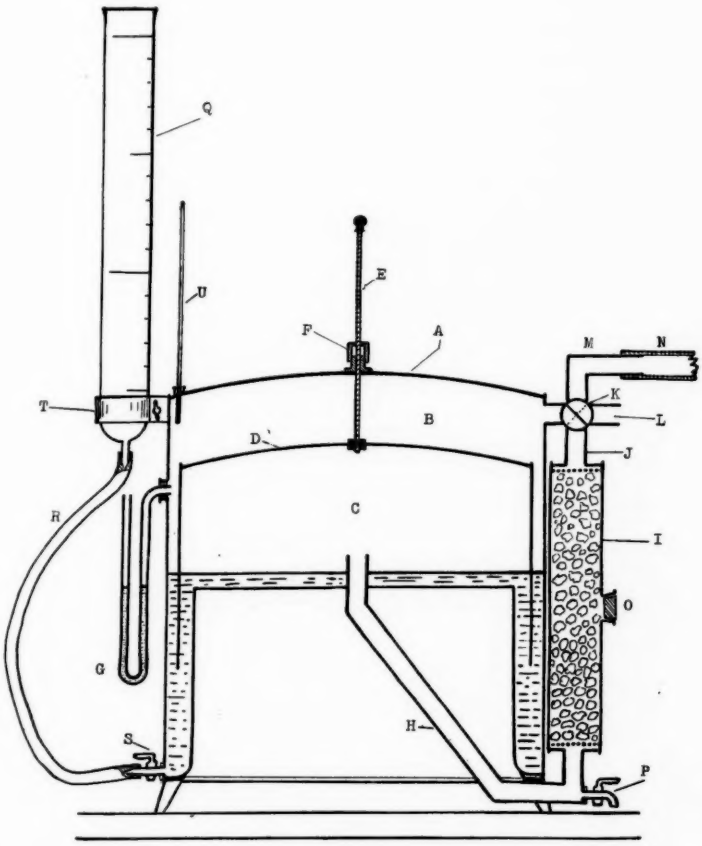

Fig. 1

A glass manometer of conventional design is provided at (*G*) to make it possible to see when the pressure inside the apparatus is identical with atmospheric.

The compartment (*C*) is open through tube (*H*) to a container for soda lime or other absorbent of carbon dioxide, and thence through tube (*J*) to the four-way valve (*K*). The soda lime container (*I*) is provided with screens at each end and an opening with a suitable gas-tight stopper at (*O*). The cock at (*P*) is provided to drain the bottom of the tube (*H*) of any water spilled from (*C*) or of any liquid alkali from (*I*), and to facilitate the adjustment to atmospheric pressure subsequently described.

A graduated glass cylinder (*Q*) is provided to measure the quantity of water to be added to the container through the rubber tubing (*R*) and the stopcock (*S*). An adjustable clamp (*T*) holds the graduated cylinder in position. The graduate (*Q*) is from 300 to 500 c.c. capacity and graduated in not larger than 5 c.c. divisions.

The thermometer (*U*) indicates the temperature of the gases in the container.

A metabolism measurement may be conducted as follows: The apparatus, properly filled with water and absorbent for carbon dioxide, is placed by the subject. Mouthpiece and nose clip are applied as usual, and the subject draws air from the room or through a tube from the outside air and exhales it through the tubes (*N*) and (*M*) and out through (*L*) with the valve (*K*) set as indicated. Graduate (*Q*) is filled with water to the zero mark, stopcock (*S*) closed, cock (*P*) opened, the diaphragm (*D*) raised to its highest position and the packing nut tightened with the fingers until the friction on the rod (*E*) just prevents the diaphragm from falling under the force of gravity. The contents of the apparatus will be at atmospheric pressure.

The operator watches the breathing of the subject, preferably by observing the motion of the flutter valve, and just at the end of an exhalation, he turns the valve (*K*) to the position indicated by the dotted line and starts a stopwatch. Exhaled air enters (*B*) and drives the diaphragm down displacing the air in (*C*) through the tube (*L*). The larger the apparatus, the greater the accuracy, but the author has found that twenty to thirty breaths are sufficient to get an accuracy comparable with or better than the accuracy of the recording type of instrument.

When the operator sees that the diaphragm has almost reached its lowest point, he again turns the valve (*K*) back to the original position and stops the stopwatch at the end of an exhalation. Thus a definite number of breaths are accumulated in a measured time. The carbon dioxide exhaled in this measured time may be estimated as follows:

With the valve (*K*) left in the position indicated by the heavy line, the cock (*P*) is left open for a few moments while the air is coming to constant temperature. When the cock is closed, the contents of the apparatus are left at atmospheric pressure. If the gasometer diaphragm is lifted, the exhaled air in space (*B*) will be driven to space (*C*) through the absorbing substance and the carbon dioxide will thus be removed. Several strokes of the diaphragm will insure the complete removal of the carbon dioxide. This removal will lower the pressure, and during the absorption the cock (*S*) must be opened and water run in to take the place of the carbon dioxide and keep the pressure at atmospheric.

When the absorption is complete, the diaphragm is lowered to the bottom, the position at the start of the absorption, and the apparatus is left standing for a few moments while the temperature becomes constant again and while any water vapor which may have been removed by the absorbent is being replaced by evaporation. Water is then run in or out of the graduate (*Q*) until the manometer indicates that the pressure is atmospheric. With water in the manometer, this adjustment may be made very accurately, and the amount of carbon dioxide read

from the graduate with an error of less than one per cent. The fall of water in the graduated cylinder (Q) is measured in cubic centimeters and is taken as the volume of the carbon dioxide exhaled in the measured time and absorbed in the apparatus at atmospheric pressure. No correction is necessary for water vapor, since the space is saturated at the start and at the close of the determination, and leakage is avoided since the contents of the appartus is never far removed from atmospheric pressure at any time during the determination.

The author has made comparison determinations of basal metabolism using this device and an instrument of well-known make having a kymograph oxygen-recording equipment. The following data were obtained on one comparison run:

TABLE I

DATA OBTAINED OCTOBER 4, 1931.

SIMPLIFIED INSTRUMENT		RECORDING INSTRUMENT	
Temperature	= 26°	Temperature	= 26°
Pressure	= 764 mm.	Pressure	= 764 mm.
CO_2 volume	= 200 cc.	O_2 Rate from Kymograph	= 1.68 liters
Time	= 1 min. 8 sec.	For Time	= 8 min.
CO_2 Rate/hour	= 10.60 liters	O_2 Rate/hour	= 12.6 liters
Using RQ = 0.82 and correcting for CO_2 in the air the O_2 rate was calculated to be	= 12.77 liters		
Calories/sq. meter	= 34.8	Calories/sq. meter	= 34.3
Difference = 1.45 per cent			

It will be observed that the instrument described gave a value 34.8 calories per square meter where the recording device gave 34.3 calories per meter. On inspection of the kymograph record, it was found that the extent to which the slope of the breathing line could be estimated may have been in error by as much as the difference observed. Furthermore, the time required for the recording was eight minutes, while the time for the simplified determination was only one minute, eight seconds. Only fifteen breaths were collected, yet the accuracy compares favorably with the recording method. The reason for this is obvious: the irregularities of breathing were recorded in one instrument, averaged in the other.

This brings up the question of the most convenient size. The author regards a minute and a half as a minimum time for collecting a sample, although he has demonstrated that a little over a minute will give results comparable with the recording method. This means a minimum of twenty to twenty-five breaths, and demands a container at least fourteen inches in diameter and thirteen to fifteen inches high. Larger instruments are more accurate and would not be found inconvenient even for those desiring a portable apparatus. It would not be impractical to make the apparatus as large as the desired accuracy demands. Furthermore, the process of measuring the carbon dioxide contained in twenty to thirty breaths (approximately 200 to 500 c.c.) to an accuracy of 2 c.c. has its advantages over a recording of larger volumes with larger errors in that the subject is subjected to less inconvenience, and it is possible to make two determinations and average the results in the time required to make one measurement of equal accuracy by the oxygen-recording method.

Another advantage to be derived from the simplified method is in the elimination of the need of pure oxygen. The subject breathes air, and if that is drawn through a tube from outside a window, the carbon dioxide content will be too small to introduce much error even if neglected. However, it is a simple matter to make a blank determination of the carbon dioxide in the air and let the subject breathe from the air of the room. Other advantages are the simplicity of the operations and the lack of complicated parts and adjustments. Enough soda lime may be provided to make fifty to seventy-five determinations without attention. The manipulation is as simple as a freshman chemistry experiment, and the calculations may be made from the ordinary charts for the purpose.

The author is not able to give an accurate estimate of cost at the present time, but the facts that a ten-dollar stopwatch may be substituted for a kymograph recording device, and that the rest of the apparatus is simple in proportion, indicate that the device could be made at a reasonable figure.

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MODIFICATION OF USE OF WRIGHT'S STAIN*

By F. D. LaRochelle, M.D., Springfield, Mass.

UP TO the time of the appearance of Schilling's book "Das Blutbild und Seiner Ververtung" in 1929 we were using Jenner's and Wright's stain for blood smears interchangeably with a slight preference for the latter. These had for years given fair results in a number of instances, but neither compare with the results obtained with procedures involving the Giemsa stain. On trying out the technics as described by Schilling, and Schleip and Alder "Atlas der Blutkrankheiten" we obtained good results, but found the process an exceedingly delicate one, and we soon discovered that this delicacy was not due to the stains themselves but rather bound closely with the hydrogen ion concentration of the water. The results from Giemsa stain were so good that we endeavored to simplify the technic, and by making a few modifications, we have been able to obtain with great ease smears that are in every way equal to the best results obtained from Giemsa technics. The technic here described aims at reducing the chances of variations in concentration to a minimum.

The microscope, light, filters, stains, glassware, and especially the water must all be brought to an optimum condition, and this of necessity demands time and attention; but the results are well worth the effort.

The glassware must be clean and of the best quality, and unusual precautions should be taken to prevent contamination with acids or alkalies or other chemicals. The purpose of the technic here described is to make these precautions more or less unnecessary, but they should at least be kept in mind, and if good results are not obtained, it must always be remembered that the water is usually the factor responsible.

*Received for publication, September 22, 1931.

Naturally smears must be well made; we usually follow the technic described by Schilling although we find slides about as satisfactory as cover-glasses for spreading the blood.

As a rule the time necessary to travel from the patient to the laboratory is sufficient for the smear to dry in air; five minutes is a satisfactory time in an ordinary room. Under no circumstances is heat to be used throughout the process; the small advantage gained is more than offset by the danger of damaging the blood.

TECHNIC

Once the smear is dry it is placed over a water glass whose margin must describe an exact horizontal plane if the stain is to flow evenly. Wright's stain is then added, 30 drops is the usual quantity, to cover the smear and in two or three minutes an equal quantity of specially prepared water is added.

Preparation of the water is the important step. We formerly used distilled water but now we prefer tap water. To prepare the water a gallon from the tap is drawn into a clean bottle and a smear stained in the usual way to determine the hydrogen ion concentration; our water is too acid and requires about 5 drops of 5 per cent NaoH per gallon. This varies of course and must be adjusted for every source. A few smears are then stained and NaoH added drop by drop until the desired tint is obtained. In some localities it might take less than 5 drops. But the important point to remember is that bad results come from the water. Once the proper hydrogen ion concentration is found other factors such as stains, time, etc., are of secondary importance and excellent smears are obtained with little care and few failures.

The combination of Wright's stain and water is left on for two or three minutes and to this mixture is added 5 to 6 drops of Giemsa's stock solution and mixed rapidly by tilting the glass; as a rule six to eight minutes is the best time to stain but this may be decreased or increased according to circumstances without material changes in the end-result. In our instance this procedure intensifies somewhat the basic stain and this is offset by washing in tap water that is slightly acid. Naturally the use of tap water for washing is a great convenience.

The smear is then dried in air by standing it on end and examined with oil. We have found liquid petrolatum preferable to cedar oil for this purpose.

This technic may appear complicated but really it is not and can be learned by the average student technician in a few hours, and to us has given more uniform and better smears than any technic used previously.

SUMMARY OF TECHNIC

1. Cover smear with Wright's stain, about 30 drops, two to three minutes.
2. Add equal quantity prepared water.
3. Add 5 to 6 drops Giemsa's stock solution for six to eight minutes, according to shade desired.
4. Wash with tap water.
5. Dry in air.
6. Examine with liquid petrolatum.

THE SPRINGFIELD INFIRMARY.

THE ESTIMATION OF BILIRUBIN IN BLOOD SERUM*

By Marjorie Pickens, B.A., and L. Bauman, M.D., New York City

THE determination of bilirubin in serum has become a routine practice in the hospital in cases of jaundice. The quantitative colorimetric estimation of van den Bergh depends on the formation of an azo dye through interaction of bilirubin and diazotized sulphanilic acid. The originator at first employed a diazotized solution of bilirubin itself as a standard of comparison. Bilirubin, however, is very expensive, difficult to obtain in pure form, and is readily oxidized to a green pigment when in solution. For these reasons van den Bergh suggested the use of an ether solution of standard ferric thiocyanate which has a color comparable with that of azobilirubin. In our hands this salt has been preferable to the cobalt solutions more recently recommended.

In the literature we were unable to find a comparison of results when both bilirubin and iron standards were used on the same sample of blood. For a considerable time we were doubtful of the accuracy of the method when the substitute was used. The likelihood of evaporation of the volatile solvent while in the colorimeter cup and the difficulty of exactly matching colors seemed real stumbling blocks. Fortunately, after considerable effort, we secured some chemically pure bilirubin. The results which follow were obtained on the same sample of blood serum with the two standards.

Results Obtained When Using the Two Standards on the Same Sample of Blood

BILIRUBIN mg. %	FERRIC THIOCYANATE mg. %	BILIRUBIN mg. %	FERRIC THIOCYANATE mg. %
4.4	4.9	13.4	13.0
2.3	3.2	5.3	4.3
2.7	3.2	3.5	3.3
3.1	3.2	13.9	14.0
5.7	4.2	3.7	3.2
3.9	3.6	11.8	11.1
1.4	1.5	12.9	13.0
1.4	1.5	8.9	9.7
2.8	2.1	3.5	3.2
2.8	2.1	11.8	10.0
2.0	2.1	10.5	9.5
15.5	15.4	3.7	3.4
13.6	13.5	6.2	6.0
19.9	21.3	6.6	7.4
3.9	4.6	10.1	10.9
9.0	11.1	4.6	5.3
9.4	10.9	9.0	9.3
14.5	12.5	3.1	2.9
9.1	9.2	5.5	4.6
9.6	8.8	6.7	6.5
5.2	4.5	6.8	6.9
11.7	12.8	11.3	10.6
7.9	7.6	3.8	3.6

*From the Chemistry Laboratory, Department of Surgery, Presbyterian Hospital and Columbia University.
Received for publication, October 3, 1931.

The methods employed were those of Greene, Snell, and Walters[1] for the serum, while the bilirubin standard was prepared and treated according to Chiray and Thiébaud.[2]

The results, while not sufficiently accurate for scientific work, may be regarded as acceptable for ordinary clinical purposes.

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A DEVICE DESIGNED TO SIMPLIFY THE HANDLING OF CELLOIDIN SECTIONS*

By Morris Mass, B.S., and Nathan Schaffer, B.A., M.D., Newark, N. J.

THERE are distinct disadvantages in the present methods of handling celloidin sections. With the method in vogue, after the section has been cut and placed in 80 per cent alcohol, it is carried through the various solutions by means of a glass rod flattened at one end. The section curls about the glass rod and in order to free it, a small amount of agitation must be produced. In so doing, the tissue tears and separates from the celloidin sheet. Since agitation is repeated in each of the fluids, tearing is an invariable result at some stage.

Again when the section is transferred from the eosin into the concentrated alcohols, a marked amount of surface tension is set up and this too causes tearing. When the section is carried into the clearing fluid, the surface tension is still greater, the section is torn still more and in some instances destroyed.

Only expert technicians can obtain good results with the present method and often after a certain amount of failures. The work of lifting the sections with a glass rod, and of only running through one section at a time makes the process very tedious. This is especially true of eye work where serial sections are desired. The time needed to run through twenty sections is approximately five hours and constant care must be practiced throughout.

We have devised a simple apparatus to overcome the above disadvantages. This apparatus is easily constructed and easily handled. The materials consist of ordinary copper window mesh, lead solder and small numbered tabs, as are used in numbering animals. Squares, one and one-half inches in length, are cut out of the copper mesh, the edges of which are strengthened with a thin border of lead solder. The small numbered tab is soldered to one corner. Two squares are hinged together using either a strand of wire from the mesh, or by making a small hinge out of fine tubing and an ordinary pin. (Fig. 1.) A small clip is made out of a piece of flat metal. (Fig. 1.) In order to facili te handling of a number of these frames a carrier is made, utilizing one of the squares and soldering wire to the edges.

*From the Clinical and Pathological Laboratories Newark Beth Israel Hospital.
Received for publication, November 7, 1931.

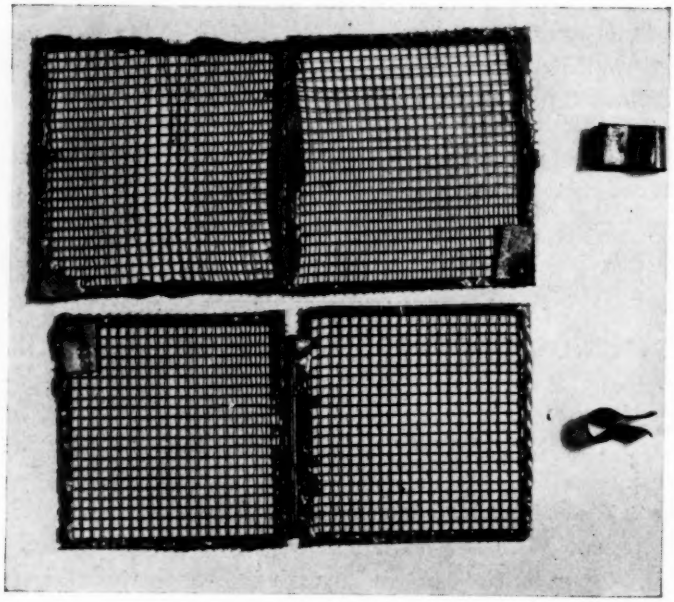

Fig. 1

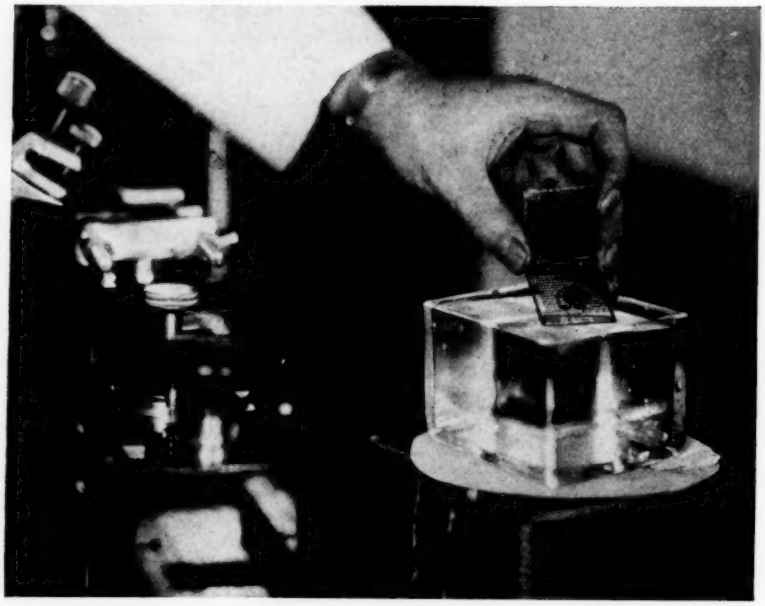

Fig. 2

When the section has been cut and placed in 80 per cent alcohol, it is floated onto an open frame, held between the thumb and first two fingers of the left hand. The section is kept smooth by means of a fine brush held in the right hand. (Fig. 2.) It is then secured by closing the frame and attaching the clip. The closed frame is now placed into the 80 per cent alcohol until needed. There is no danger of any confusion as these frames are serially numbered. The loaded frames are placed on the carrier and dipped into the different solutions. By virtue of their mesh construction, the fluid drains easily from the frames and the carrier, and the latter may be tapped lightly against the sides of the vessels

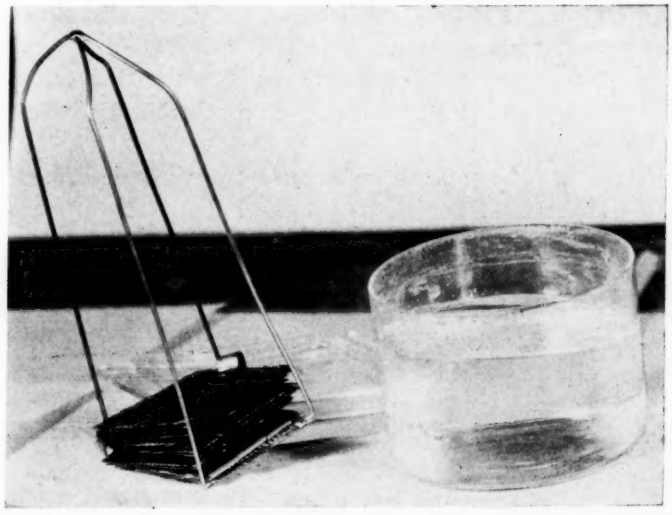

Fig. 3

without injury to the tissue. (Fig. 3.) Lastly, the frames are placed into the clearing fluids a few moments, opened, and the sections floated out onto the respectively numbered slides and mounted.

The advantages of this device are readily seen. Manipulation and surface tension are reduced to a minimum. There is little overlapping and wrinkling of the section on the slide. The staining and mounting may be done by an inexperienced technician with no danger of damage to the tissue. The time necessary to run through twenty sections is one-fifth that used in the old method. As many sections may be done at one time as there are frames made. In eye work this device is invaluable, not only because the above disadvantages are eliminated, but the lens is preserved intact.

A CYANHEMATIN STANDARD FOR THE SAHLI HEMOGLOBINOMETER*

By Robert D. Barnard, Chicago, Ill.

THE use of pure hemin as a standard for hemoglobinometry has recently been reported by Elvehjem (1931). Such a step may be considered an advance over other methods for preparation of standards, at least in those laboratories where gasometric determinations of oxygen capacity are not available. With pure hemin, standards may be weighed out; can be renewed as often as is deemed necessary and form a basis of comparison attainable by widely separated laboratories.

Since 1930, we have been using a standard of cyanhematin prepared from a weighed sample of hemin. Laked, hematinized blood is diluted with cyanide solution and compared with this standard in the Sahli colorimeter.

SOLUTIONS REQUIRED

1. The standard is made by dissolving 64 mg. of crystallin hemin, prepared by the method of Chalfejew† in 1 liter of 1 per cent sodium cyanide solution.

2. A solution of approximately 0.05 molar hydrochloric acid, to which a few drops of caprylic alcohol are added. This solution lakes the blood and converts its hemoglobin to acid hematin. The caprylic alcohol prevents the frothing which is ordinarily troublesome when working with the Sahli colorimeter.

3. A solution of 1 per cent sodium cyanide. This solution is used to dilute the acidified blood in the colorimeter chamber as it converts the acid hematin to cyanhematin.

PROCEDURE

The standard tube of the Sahli instrument is broken off close to the top and the acid hematin suspension is discarded. The tube is then cleaned and filled with the cyanhematin standard (Solution 1). (The original solution prepared in this laboratory has kept its color value for over one year.) The tube may be sealed in a flame or with a small paraffined cork.

For the dilution of the blood sample, the original directions for the use of the Sahli instrument are followed, with the exception that the final dilution is made with cyanide solution. The colorimeter tube is filled to the mark "10" with 0.05 molar hydrochloric acid (Solution 2). Blood is drawn into a 20 c.mm. pipette, the tip is wiped dry, and then placed directly under the level of the hydrochloric acid in the chamber. If this is done carefully, the corpuscles will

*From the Clinical Laboratory of the Chicago Free Dispensary and the Department of Physiology of the Chicago Medical School.
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†500 c.c. of defibrinated or citrated blood is added to 2 liters of glacial acetic acid which has been heated to 80° C. As soon as the temperature has fallen to the neighborhood of 55° the liquid is again heated to 80°. The crystals which should form at once are allowed to settle, washed with several changes of distilled water, then with alcohol and finally with ether, after which they are dried at 115° C.

form a layer beneath the acid and the pipette walls may be rinsed in the clear supernatant fluid.

The contents in the chamber are mixed so that hemolysis occurs. When a deep brown color results, 1 per cent sodium cyanide (Solution 3) is added, drop by drop, until a color comparison is secured.

COMPARATIVE DETERMINATIONS OF HEMOGLOBIN CONCENTRATION

NEWCOMER METHOD	CYANHEMATIN METHOD
13.75	13.36
13.60	13.54
15.90	15.30
12.30	12.48
12.30	12.17

CALCULATIONS

The value of "100" on the scale of the Sahli instrument with the cyanhematin standard herein described, represents a blood sample which contains 1 millimol of iron or 16.67 gm. of hemoglobin in 100 c.c. of blood. To determine the gram percentage of hemoglobin from the reading of the instrument, it is necessary only to divide the latter figure by 6. For example, if the color comparison is secured when the meniscus of the fluid in the color chamber is 72, the gram percentage of hemoglobin is $72 \div 6 = 12$. The arbitrary normal of 16.67 is chosen because it represents an even decimal fraction of the molecular equivalent of hemoglobin and is at the same time close to the figure given by Newcomer for the average (16.93) content in human blood.

It is felt that certain disadvantages, ordinarily ascribed to the Sahli hematometer have been overcome by adoption of the cyanhematin standard. The advantages of the present method are:

1. The simplicity of the original instrument is retained.
2. The colloidally dispersed hematin, with its complex absorption bands is replaced by a true solution with but one absorption band in the visible spectrum. Theoretically, the latter color will more strictly conform to Beer's law and practically, it is easier to match.
3. The final color value of cyanhematin is attained instantly.
4. It is possible to complete a hemoglobin determination on blood which has inadvertently become clotted in the pipette. Such a sample may be digested with pepsin and hydrochloric acid and then converted to cyanhematin.

REFERENCES

Elvehjem, C. A.: The Preparation of Standard Acid Hematin Solutions From Hemin, J. Biol. Chem. **93**: 203, 1931.

A TRANSPARENT RULE FOR MEASURING BASAL METABOLISM GRAPHS*

B. S. Levine, Ph.D., Chicago, Ill.

THIS paper presents a description of a simple transparent rule for measuring basal metabolism graphs. After two years' experience with this rule, I believe that its advantages are threefold: first, it enables the use of unruled paper with the Sanborn-Graphic, Sanborn-Benedict and the Roth-Benedict apparatus; second, it can be used in measuring the graphs obtained with any of the machines just mentioned; and third, it gives the final readings directly and simultaneously in terms of cubic centimeters of oxygen consumed per minute and calories of heat radiated per hour. No matter which apparatus is employed, the calculation of the final results is considerably simplified, since the tables compiled for the use with the Roth-Benedict machine can be used with the graphs obtained by either of the two Sanborn apparatus, and vice versa.

The reduced photographic reproduction of the rule shows its simplicity at a glance (Fig. 1).

The relationship between the scale measurements of the rule and the distances of the intersection points, described below, is a trigonometric one; the rule may, therefore, be enlarged or reduced from its original size without in any way disturbing its general applicability. The most suitable size is one of from 8 to 10 inches square.

In using the rule proceed as follows: Place a plain white sheet of paper around the recording drum, securing it by means of small rubber bands. This eliminates the need for pasting and consequent cutting of the recording paper. Prepare the patient and obtain the graphs in duplicate following the usual technic prescribed for the particular apparatus employed. The record should extend over not less than six minutes. Upon the completion of the test period and before removing the drum, make a vertical line with the writing pen by moving it up and down at the middle of the paper, thus dividing the paper into two parts through its entire length; or make an horizontal line by resting the pen in the middle of the paper and turning the drum one complete revolution, thus dividing it into two parts through its entire width. Either of the lines thus made may serve as a base line in the application of the rule.

Remove the graph, place it on a flat surface, and in the manner usually prescribed, draw a straight line through the apices of the respiratory excursions, extending this line along the entire width of the paper.

Apply the rule as follows: Place the rule over the graph so that its vertical or horizontal guide line completely coincides correspondingly with the vertical

*From the Clinical Laboratory, Public Health Institute, Chicago, Ill.
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or horizontal base line on the graph. Now, move the rule up or down, along the vertical line, or to the right or left along the horizontal line until point of intersection *SG* (if the Sanborn-Graphic is used) or point of intersection *SB* (if the Sanborn-Benedict is used), or point of intersection *RB* (if the Roth-Benedict is used) falls upon the line running along the apices of the respiratory excursions, taking care all the while that the guide line and base line remain absolutely coincident. Note where the respiratory excursion line on the graph intersects the scale line of the rule and read the results by noting the measurement on the right of the scale line, which gives the number of cubic centimeters

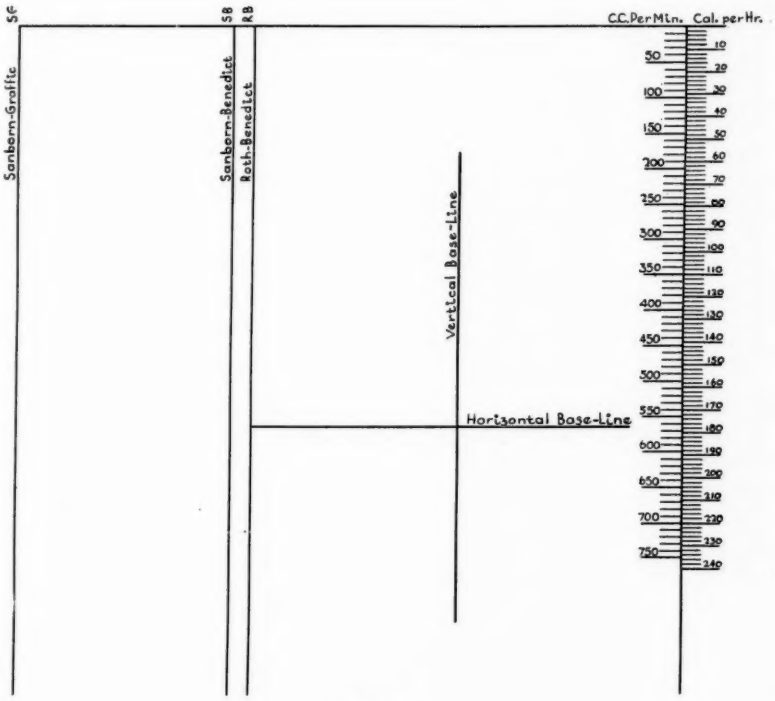

Fig. 1

of oxygen consumed per minutes, or the measurement on the left, which gives the number of calories consumed per hour.

The final results include corrections for 0° and 760 mm. pressure. Corrections for actual temperature, pressure, age, and sex, as well as the final percentile calculation, can be accomplished with the aid of the tables given in either the Sanborn or the Collins booklets, or the calculations may be completed by the method of Campbell.[1] My experience of many years has indicated to me that in making correction calculations the laboratory technician is more adept with tables than with graphs. By the use of the rule described in this paper and with the aid of either the tables compiled for the Sanborn or Collins apparatus a complete B.M.R. calculation can be carried out in as short a time as one minute.

SUMMARY

A transparent rule is described for measuring basal metabolism graphs which entirely eliminates the need of the expensive heavily ruled paper used heretofore. It shortens the process of calculation by giving final readings in terms of cubic centimenters of oxygen consumed per minute and calories radiated per hour. The rule can be made of translucent paper, celluloid or any other transparent material.

REFERENCE

1. Campbell, Walter R.: Nomograms for Metabolism Estimations, J. LAB. & CLIN. MED. **16**: 1113-19, 1931.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ANAPHYLAXIS: Prevention of Shock, Waldbott, G. L. J. A. M. A. **98:** 446, 1932.

The following conclusions are drawn from a study of eight fatal cases:

1. A negative history of personal or familial allergy or of previous serum injections should not be relied on in ruling out the possibility of serum sensitization.

2. Skin or conjunctival tests should be performed before administering serum, but a negative test does not definitely rule out sensitization.

3. Intravenous injections should be avoided as much as possible. Withdrawal of the syringe for evidence of blood may protect to some extent against accidental puncture of a vein but does not with certainty prevent this possibility.

4. If no vein is punctured, the rapid appearance of a marked local reaction should invite caution. Epinephrine administered above the site of injection and application of a tourniquet may then aid in blocking absorption.

5. Desensitization, according to Besredka, at short intervals is not a safe procedure for prevention of shock.

6. Epinephrine, if given after symptoms have arisen, does not necessarily protect against the fatal outcome of shock. In cases suspected of sensitization, it should be given admixed with the antigen.

ANEMIA, PERNICIOUS, Macrocytosis and Erythrocytes and Achlorhydria In, Haden, R. L. J. A. M. A. **98:** 202, 1932.

An increase in size of the average erythrocyte, best indicated in terms of volume, is the most constant and characteristic finding in the blood in the presence of pernicious anemia. An increased volume index was found in every patient in this series.

Free hydrochloric acid is seldom, if ever, found in the gastric contents of a patient with idiopathic pernicious anemia. An achlorhydria was demonstrated in every one of the 152 patients in this series.

The mean corpuscular volume may be quite large even with a relatively high count; therefore it does not vary with the red cell count.

If the deficiency which is responsible for the disease is adequately supplied, the cells return to normal size. The first indication of a relapse or a lack of a sufficient quantity of the missing principle is an increase in the volume of the red cells.

Macrocytosis may occur in the presence of conditions other than pernicious anemia but was found only 9 times in a study of 411 patients and normal individuals.

Achlorhydria is a frequent finding in various clinical conditions, especially in the age period in which pernicious anemia is most common.

A combination of macrocytosis of the erythrocytes and achlorhydria is seldom if ever found, except in the presence of pernicious anemia.

The finding of an absence of free hydrochloric acid on gastric analysis and an increased mean corpuscular volume or plus volume index is a practically constant finding and one that is necessary for the diagnosis of active pernicious anemia; if demonstrated, it is almost pathognomonic of the disease.

AGRANULOCYTOSIS, Experimental, Fried, B. M., and Damashek, W. Arch. Int. Med. 40: 94, 1932.

The purpose of the present study has been to determine the possible similarity between the blood picture as seen in agranulocytosis in man and that found in a form of experimental sepsis in rabbits.

The results have shown that there are close similarities between the agranulocytosis resulting from the hematogenous infection of rabbits with Salmonella suipestifer and that observed in cases of agranulocytic angina in man. Thus, the reaction in severe cases of human agranulocytosis corresponds to that of the animals that received overwhelming doses of bacteria; i. e., a persistent neutropenia and an intense necrosis of the bone marrow without signs of regeneration. A close similarity likewise exists between the "recovery phase" seen in the circulating blood in clinical agranulocytosis and that disclosed in the circulation of rabbits that were infected with relatively small doses of bacteria; i. e., a marked histiomonocytosis.

Incidentally, Schilling's clinical concept of "regenerative" and "degenerative" types of polymorphonuclear "shift" was confirmed by these experiments.

LANTERN SLIDES, A Simple, Quick, and Inexpensive Method Preparing, Reid, P. E. Science, **74**: 418, 1931.

Take a plain glass lantern slide, thoroughly clean it and allow to dry. When the slide has become quite dry apply a thin coat of albumen from a fresh egg and again allow the slide to dry. A smooth brush is essential in getting the coat of albumen evenly placed to avoid a streaked appearance when done.

As soon as the albumen has completely dried, place the plate with its coated side uppermost, over the diagram or other copy, and trace on the slide with India ink. The width of the lines may be varied by using pens of different sizes. Colored effects may be added in the same manner except that inks made from aniline dyes (such as the common writing fluids) should be used. The pigmentation in colored India inks make them all appear black on the screen.

Mount in the usual manner after the ink has dried by placing the newly made plate face down on another clean slide and fastening together with the usual lantern slide material or with adhesive tape.

These slides are not temporary as might be supposed but may be left in the lantern for long periods of time without injury in spite of the intense heat of some lanterns.

BACTERIA, Pure, Smooth and Rough Colonies at Will, Quirk, A. J. Science **74**: 461, 1931.

Make a needle transfer of the culture (broth, agar slant, or diseased material) to a P_H 6.0 and a P_H 7.0 beef infusion broth tube. Then make a second dilution tube from each P_H grade of the (1) seeded broth tube. Hold the (2) dilution broth tubes in both P_H grades for 18 or 24 hours at room temperature.

After this growth period again make (1) and (2) dilutions from each P_H grade of the young culture to a corresponding P_H grade of broth. From the last (2) dilution broth tube transfer to a melted P_H 6.0 and P_H 7.0 beef infusion agar tube for poured plate examination.

This modified technic produces on the plates pure culture of the S or R colonies.

This technic involves three factors necessary to assure the pure S and R colonies. They are as follows:

(a) Dilution before and after the young growth period of the organism.

(b) Young culture.

(c) P_H of the culture medium.

For the smooth colony use only P_H 7.0 medium. For the rough colony use only P_H 6.0 medium.

The S colony is virulent; the R colony is avirulent.

Attention is called to the fact that an interchange of P_H grade from broth to agar plate may result in intermediate types with a corresponding interference in demonstration of virulence and nonvirulence on the host plant.

TUBERCULOSIS, Direct Culture of B. Tuberculosis from the Blood, Kren, O., and Lowenstein, E. Klin. Wchnschr. **10**: 974, 1931.

Preparation of Culture Medium: Five unbroken eggs are placed in 5 per cent solution of washing soda for 30 minutes, then rinsed in running water for 20 minutes, then soaked

in 1:1000 bichloride solution for 20 minutes and again rinsed in water, and finally with 70 per cent alcohol.

The following solution is prepared:

Mono-potassium sulfate	1.0 gram
Sodium citrate	1.0 gram
Magnesium sulfate	1.0 gram
Asparagin	3.0 gram
Glycerine	60.0 c.c.
Distilled water	1000.0 c.c.

To each 150 to 160 c.c. of this solution add 6 grams of potato-meal and 12 c.c. of glycerine. This mixture is boiled for 15 minutes and then placed in a 56° C. water-bath for 1 hour with frequent shaking.

The entire contents of four of the prepared eggs are now added and also the yolk only of the fifth egg. The mixture is then shaken vigorously with glass beads and 5 c.c. of 2 per cent sterile aqueous solution of Congo red. The medium is then filtered through sterile gauze and distributed in sterile tubes.

The tubes are placed in a thermostat in a slanting position and the medium coagulated after which it is sterilized in the Arnold for 1 hour on two successive days. The tubes are finally incubated 48 hours at 37° C. as a check of their sterility.

Collection and Inoculation of Blood: From 8 to 10 c.c. of blood are drawn by venipuncture with a syringe into which 2 c.c. of sterile 5 per cent aqueous solution of sodium citrate have previously been drawn.

The blood is expelled into a sterile tube, centrifuged, and the plasma drawn off and discarded by means of a sterile pipette.

To the sedimented corpuscles add 5 c.c. of sterile 5 per cent acetic acid and stir with sterile precautions and again centrifuge. Withdraw the supernatant fluid with a sterile pipette and wash the mass twice with sterile distilled water.

The final sediment is now distributed over the surface of 4 to 6 slants being well spread and the tubes allowed to stand until the film of inoculum has dried. The tubes are then sealed with sealing wax and incubated at 37° C. Strictly aseptic technic must be observed throughout the whole procedure.

If the blood is received in a nonsterile condition it should first be shaken with 15 per cent sulphuric acid, allowed to stand 5 minutes, washed twice with sterile water, and the sediment thus obtained used for inoculation.

PNEUMOCOCCI, Immediate Typing of, Armstrong, R. R. Brit. M. J. 3703: 187, Jan. 30, 1932.

A method is described whereby pneumococcus typing may be carried out directly from the sputum.

A Gram-stained smear is first examined to determine that pneumococci are present and also to gain an idea of the general character of the bacterial flora.

Selected flakes of sputum are then placed on each of four slides marked I, II, III and C (control).

A large drop of the respective type serum is placed next to the sputum, a drop of normal saline being used for the control. The sputum is then mixed with the serum, a cover slip applied and the preparations are allowed to stand while the Gram preparation is being examined.

Positive reactions are very striking and consist of a marked increase in the size of the pneumococci which also take a ground-glass appearance and appear surrounded by a highly refractive peripheral zone.

The reaction may sometimes be delayed for as long as 15 to 20 minutes but is highly accurate when checked by the more elaborate methods.

The accuracy of the method is corroborated and highly commended by Logan and Smeall (Direct Method of Typing Pneumococci, Logan, W. R., and Smeall, J. J., Brit. M. J. 3703: 188, Jan. 30, 1932).

Because pneumococci may be unevenly distributed in the sputum these latter authors, however, prefer to conduct the test with a saline emulsion of the sputum.

MUSEUM TAGS, Chemical Proof, Schmidt, K. P. Science, **73**: 1931, 23, 1932.

The paper known as Dennison's fiber-proof paper, was devised especially as a chemical-proof paper for laundry tags. It appears to be a paper impregnated with albumin, which is subsequently hardened by treatment with formaldehyde. This paper comes in 20″ x 24″ sheets, somewhat variable in thickness. It does not soften in water, alcohol or formalin solution.

The 20″ x 24″ sheets in practice, are cut into ⅞″ strips. These are printed with rules set ¼″ apart. Numbers are then stamped into the paper, to a depth of about half the thickness of the stock, by means of an automatic numbering machine. The printed rules serve as guides, so that the finished tag measures ¼″ x ⅞″. These impressed numbers are then inked by hand with Higgins waterproof drawing ink, to increase the legibility of the numbers, and dried. The numbering machine perforates the strip opposite each number, and the number strips next have the strings attached. The individual tags are then cut from this strip as wanted.

TUBERCLE BACILLI, Stain for Non-Acid-Fast Bacilli and Granules, Alexander, E. G. Science **75**: 1937, 197, 1932.

Smears are stained with carbol-fuchsin and decolorized as usual after which one of the counterstains described below is applied.

COUNTERSTAIN METHOD I

Flood the smear with 8 drops of Loeffler's methylene blue. Add immediately 6 - 8 drops of 0.05 per cent NaOH from a medicine dropper. Move the slide gently from side to side to mix, and let stand two to three minutes. Wash with tap water, dry and examine under the oil immersion lens. The contrast between the red acid-fast tubercle bacilli and the blue non-acid-fast tubercle bacilli is striking.

COUNTERSTAIN METHOD II

Flood the smear with 8 drops of 1 per cent aqueous crystal violet solution. Add immediately 6 - 8 drops of 5 per cent $NaHCO_3$. Move the slide gently from side to side to mix, and let stand not more than 2 minutes. Wash in tap water, apply Gram's iodine for 2 minutes, wash, and decolorize 20 - 30 seconds with a mixture of equal parts of acetone and 95 per cent alcohol; wash, dry, and examine under the oil immersion lens. The non-acid-fast bacilli appear violet. In addition the "Granules" in the red acid-fast bacilli and in the violet non-acid-fast bacilli stand out prominently as violet-black bodies.

MONONUCLEOSIS, INFECTIOUS, Presence of Heterophile Antibodies In, Paul, J. R., and Bunnell, W. W. J. M. Sc. **183**: 90, 1932.

The following technic was used.

The authors have employed the methods used by Davidsohn for determining the presence and titer of sheep cell agglutinins and hemolysins. The technic is quite simple.

Sheep Cell Agglutinins: Sera, obtained as for a Wassermann test, were inactivated for 15 minutes at 55° C. Dilutions of inactivated sera, ranging from 1 to 4 to 1 to 32 (or higher if the heterophile antibody content was suspected to be present in unusual concentration) were set up in 0.5 c.c. portions. To these 0.5 c.c. of a 2 per cent suspension of sheep cells were added, followed by 1 c.c. of salt solution, thus bringing the total volume in each tube to 2 c.c. The test tubes were shaken and placed in the water-bath at 38° C. for 1 hour, left in the icebox overnight and on the following morning were read, after each tube had been inverted three times with its mouth covered by the finger tip.

For the sake of conformity with previous work, the readings have been recorded in terms of the original dilution of the 0.5 c.c. of sera added to each tube. With the subsequent addition of 0.5 c.c. of the suspension of sheep cells and 1 c.c. of saline, the dilution of serum becomes much higher so that actually the tube designated 1 to 4

contains 0.12 c.c. of serum in 2 c.c. of cell suspension; that designated 1 to 8 contains 0.06 c.c. of serum in 2 c.c. of suspension. Legends for recording the readings are given in the following terms: +++ Firm disk; ++ disk easily broken into large flakes; + fine agglutination; ± barely perceptible, but definite agglutination.

Sheep Cell Hemolysins. In the observations recorded below all of the results have been given in terms of sheep cell agglutinations. Sheep cell hemolysins were found to parallel the agglutinin content with such regularity that the former have not been recorded. In many of the earlier determinations both tests were run and were found to serve as a rough check, one for the other.

Hemolysin tests were run as follows: Original dilutions of 0.5 c.c. of inactivated sera, similar to those used in the agglutinin tests, were employed. To these 1 c.c. of guinea pig complement in a dilution of 1 to 30 was added, then 0.5 c.c. of a 2 per cent suspension of sheep cells, followed by 1 c.c. of saline, thus bringing the total volume to 3 c.c. in each tube. The tubes were shaken, placed for 1 hour in a water-bath at 38° C. and read.

They conclude from their studies that:

1. Heterophile antibodies, demonstrable in the form of sheep cell agglutinins, have been recorded in rather high concentrations in the active stages of 4 cases of infectious mononucleosis.

2. Apart from cases of serum disease, and one notable exception, the authors have failed to note this finding in a large series of cases representing a variety of clinical conditions, including cases of Vincent's angina, lymphatic leucemia, and other blood dyscrasias.

3. There would seem to be two possible explanations for this finding: (1) that the unknown agent responsible for infectious mononucleosis contains the heterophile antigen; (2) that we are dealing with an example of isoagglutinin production elicited by abnormal cells, which are present either in the blood, or elsewhere, during active stages of the disease.

BRUCELLA INFECTIONS, The Endermic Reaction In, Leavell, H. R., and Amoss, H. L. Arch. Int. Med. **48:** 1192, 1932.

The following conclusions are advanced:

1. The endermic reaction is of value in the diagnosis of undulant fever, particularly in cases in which no agglutinins for Brucella are present in the blood serum and in which Brucella cannot be grown on culture of the blood, urine, stools or bile.

2. The intracutaneous test is not definitely specific. Although the result is generally positive in undulant fever, it is not frequently positive, or highly suggestive, in controls.

3. Representative strains of several different types of Brucella should be used in making the test. The interpretation of the test should be based on all the reactions rather than on a single one.

4. Extracts of Brucella prepared according to the methods of Lancefield and of Ando for securing the soluble specific substance gave no more specific results in our cases than did the simple saline suspensions and extracts.

5. It is probable that an extract of Brucella prepared by prolonged shaking of a saline suspension, followed by centrifugation to remove most of the organisms, gives fewer false reactions than other preparations. But such a preparation does not always give a positive reaction in undulant fever.

6. Heat-killed bacterial suspensions seem to have more specific action than bacterial filtrates.

7. It is of value to titrate the degree of the endermic reaction by using varying dilutions of suspensions of the strains being tested, as in the tuberculin reaction.

ANEMIA, PERNICIOUS, Pigment Metabolism and Destruction of Blood In, Farquharson, R. F., Borsook, H., and Goulding, A. M. Arch. Int. Med. **48:** 1156, 1932.

The authors thus summarize their studies:

1. In patients with Addison's "pernicious" anemia in a state of relapse the excre-

tion of urobilinogen is greatly increased and may be several times the normal value. In severe cases the amount excreted daily is equivalent to 8 to 13 gm. of hemoglobin, amounting in some instances to more than one-tenth of the total blood hemoglobin of the body.

2. Following liver therapy and beginning about the peak of the reticulocyte crisis, there is always a sharp decrease in the excretion of urobilinogen, which falls in a few days to a level within normal limits.

3. In cases of pernicious anemia showing little anemia, with general macrocytosis but little microcytosis or poikilocytosis, the excretion of urobilinogen is increased somewhat above a high normal value. With liver therapy the excretion of urobilinogen falls after a low reticulocyte response and is always normal when the blood picture has become normal.

4. The amount of plasma bilirubin in pernicious anemia varies in the same direction as the excretion of urobilinogen and opposite to that of the red blood cell count. It is highest in those patients who are critically ill. When it is high it falls to a normal level during the reticulocyte response.

5. In severe cases of pernicious anemia, urobilin is frequently found in the urine. Large amounts are present only when patients are very ill. Its excess then is attributable, in part at least, to altered liver function.

6. The disturbance in blood pigment metabolism in pernicious anemia is probably due to an abnormality of the red blood cells in virtue of which they suffer early destruction. The abnormal red blood corpuscles present in relapse are the ones most affected. Adequate liver treatment, promoting the production of normal red blood cells, retards the rate at which destruction of the blood occurs and allows the blood picture to become normal.

BLOOD SUGAR: Following the Rectal Administration of Dextrose, Scott, E. L., and Zweighaft, J. F. B. Arch. Int. Med. **49**: 221, 1932.

It has not been possible to demonstrate a rise in the blood sugar curve as a result of administering dextrose in retention enemas.

The slight drop that the curves show may be due to a stimulation of pancreatic activity brought about by the absorption of a slight amount of dextrose, or, more probably, to chance variation.

A variable and frequently considerable amount of dextrose administered by enema may be recovered from the stools after two and one-half hours.

TUMORS, a Coagulofloccculation Test for Malignant, Weiss, E. Arch. Path. **13**: 106, 1932.

The details of the method follow:

Glassware and Apparatus.—For the measuring of serum and antigen, 0.2 c.c. pipettes are required. For the dilution of the serum, 1 c.c. serologic pipettes are most satisfactory. Wassermann tubes with a diameter of ½ inch (1.27 cm.) and 4 inches (10.16 cm.) long appear to be very suitable. Similarly, tubes of the same diameter, but 2 inches (5 cm.) longer, are useful in instances in which deeper submersion in the water-bath is necessary or more desirable. The glassware should be rinsed with warm water and placed overnight in a cleaning solution (equal parts of a 6 per cent solution of sulphuric acid and a 6 per cent solution of sodium or potassium dichromate), then rinsed several times with distilled water and allowed to dry. Racks of Wassermann tubes with two or three rows of holes are most useful. A water-bath easily adjustable to from 54° to 55° C. and maintaining a uniform temperature is required. An interval timer with an automatic alarm is convenient on account of the brief incubation.

Blood Serum.—Serums should be fresh and thoroughly centrifugated; they do not require inactivation. Serums that are rich in lipoids, contaminated or hemolytic should be eliminated as unsatisfactory for the test.

Serum Diluent.—Distilled water is used instead of salt solution.

Antigen.—Plain alcoholic beef heart antigen: Beef heart is freed from all fat and finely ground. One hundred grams are extracted with 1,000 c.c. of 95 per cent alcohol for

three days at 37° C. and then left overnight at room temperature. The extract is then filtered and kept in dark bottles, closed with rubber corks. The antigen is then ready for use.

Sodium Chloride Solution.—Sodium chloride solution is prepared by dissolving 40 gm. of chemically pure sodium chloride in 130 c.c. of distilled water. A saturated solution of sodium chloride is equally satisfactory, if filtered before it is used.

PROCEDURE

Method of Calculating Dilutions of Serums Previous to Use in Test.—The dilution of serums is carried out according to the percentages of hemoglobin. Tallquist's scale is most commonly used in the determination of hemoglobin; it is disadvantageous because it is less accurate than other procedures. In order to have uniformity in the results obtained, Dare's hemoglobinometer is used as a standard. To the percentage of hemoglobin obtained with Dare, 10 is added, and the sum is divided by 20, which gives the dilution for the respective serum. (For instance, if the reading is 70 per cent, 10 is added, giving 80. This divided by 20 equals 4; the dilution of the serum in this case would be 1:4.) Tallquist's scales can be used if their average reading is previously compared with Dare and the difference taken into consideration in the calculation of the dilutions of the serums. (For instance, if the Tallquist scale shows a 10 per cent higher reading than the Dare, nothing is added to the hemoglobin reading before dividing with 20. If the Tallquist reading is 15 per cent or 20 per cent higher than Dare, 5 or 10, respectively, is subtracted from the reading before dividing with 20.) When the Dare reading is 40 per cent or less, the serums should be diluted only to 1:2.5.

Titration of Antigens Previous to Use in the Test.—The selection of the proper amount of antigen is based on its reaction with malignant and syphilitic serums. The syphilitic serums are more sensitive than the normal serums and are therefore more valuable for the titration. Properties similar to those of syphilitic serum are observed also in the serum in jaundice. All antigens are titrated in the following manner: In each of two rows of a rack, eight tubes are placed. Increasing amounts of undiluted antigen (0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26 c.c., etc.) are placed in the corresponding tubes of both rows. In each tube of the first row, 0.6 c.c of the diluted (according to the procedure described) malignant serum is added, and each tube of the back two receives 0.6 c.c. of the similarly diluted syphilitic serum. The tubes are thoroughly shaken and then placed in a water-bath for five minutes at from 54° to 55° C. After the incubation, the content of each tube is slowly diluted with 2.5 c.c. of saturated sodium chloride solution, and the results are recorded. Usually, the smaller amounts of antigen cause turbidity in the syphilitic and malignant tubes, "lower nonspecific zone." Larger amounts cause turbidity in the syphilitic tubes, while in the malignant tubes the more or less coagulated serum floats on the surface of the saline solution, which contains floccules. This is the "specific zone." Still larger amounts of antigen cause a positive reaction (flocculation) both in the malignant and in the syphilitic tubes, "upper nonspecific zone." The largest amount of antigen that causes only turbidity in the syphilitic tube and a distinct coagulo-flocculation in the malignant tube is selected as the proper amount for the test (= titer). The titrated amount of antigen should also be tested with icteric and anemic serums. The titer remains the same for an indefinite period if the antigen is properly preserved.

Routine Test.—Wassermann tubes are placed in two rows in the racks. The tubes of the first row are used for the main test with the unknown serums and also for the malignant, syphilitic, icteric, and anemic controls. The last tube in the first row contains the antigen control. The tubes in the second row serve as the serum controls for the unknown serums and also for the malignant, syphilitic, icteric and anemic serums. The titrated amount of the undiluted antigen is placed in each tube of the first row. The corresponding amount of distilled water is placed in all tubes of the second row. Six-tenths of a cubic centimeter of each diluted serum is added to one tube in the first row and an equal amount of the same serum to the tube behind in the second row. Six-tenths of a cubic centimeter of distilled water (instead of serum) is added to the antigenic

control. The ingredients of malignant, syphilitic and icteric controls and their serum controls should be the same as those used for the unknown serums. All tubes are then shaken and placed in a water-bath at from 54° to 55° C. for five minutes. After the incubation, 2.5 c.c. of a saturated, or 32.5 per cent sodium chloride solution is slowly added to each tube, and the results are read.

If the required amount of the unknown serum is not available, the test may still be performed successfully if the remaining constituents for the reaction are decreased proportionately.

Controls.—The following controls are necessary each time the test is carried out: (1) antigen control, (2) serum control (each serum should have a serum control), (3) malignant, syphilitic, icteric and anemic controls.

Interpretation of the Results.—The controls should be examined before making readings of the unknown serums. The malignant control should show a thick layer of coagulated serum floating on the surface of the salt solution, which contains many large floccula. All other controls should remain uniformly turbid. One tube is read for each unknown serum. Tubes showing the same reaction as the malignant control are read as strongly positive. Tubes with a distinct flocculation without showing in addition a layer of suspended coagulated serum on the surface of the saline solution are read as weakly positive. Uniformly turbid tubes are read as negative. Tubes with a doubtful flocculation are also read as negative and the test should be repeated.

Sources of Error.—(1) The used serum is hemolytic, contaminated or inactivated, or contains an excessive amount of lipoids; (2) the serum is not properly diluted; (3) some other diluent was used instead of water; (4) the antigen was not properly prepared or preserved; (5) the antigen was not accurately titrated, thus causing either nonspecific reactions or a low percentage of specific reactions; (6) the antigen was not vigorously mixed with the diluted serum; (7) fallacies occurred in the reading of the temperature and in the duration of incubation; (8) another reagent than the saturated salt solution was used for the dilution of the serum-antigen mixtures after the incubation; (9) the tubes were shaken after addition of salt solution, thus rendering the reading difficult.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr Warren T. Vaughan, Professional Building, Richmond, Va.

Ergebnisse der medizinischen Strahlenforschung*

TO THOSE familiar with German "ergebnisse" nothing more need be said. These stately annual volumes of about 700 pages (7 by 10½ inches) are of the usual high standard. They are especially well illustrated as one might expect from the nature of the subject. Volume III has monographs on the x-ray diagnosis of pulmonary ecchinococcosis and of conditions in the nose and accessory sinuses, the ear, petrous bone, bronchi, esophagus, intestine, appendix, gall bladder; also radiation and the normal skin, light and metabolism, radiation of adenoids and diathermy in gynecology. Volume IV contains ultraviolet rays and resistance to light, pigment and light resistance, erythema from light, principles of the biological treatment of carcinoma and diathermy in ear, nose and throat work. Volume V, the cascade stomach, the physics of light therapy, mutation by radiation, radium surgery, bone tumors and work with monochromatic light.

Ueber die Akute und Chronische Gelbe Leberatrophie mit Besonderer Berücksichtigung ihres Epidemischen Auftratens in Schweden im Jahre 1927**

THIS monograph reports 163 cases of yellow atrophy of the liver, 97 of which occurred in epidemic form in Sweden in 1927. There is a good epidemiological account of the epidemic and a well illustrated chapter on the pathologic anatomy of the disease. Because the disease so closely resembles icterus catarrhalis and because it may occur in epidemic form, the author feels that it must be due to some specific causative agent not yet discovered.

Allergie des Lebensalters—die bösartigen Geschwülste†

SOME years ago von Pirquet observed that in his pediatric clinic in Vienna there were many more girls than boys under fourteen years of age who showed tuberculous infection. Wondering if this were true generally, he turned to a report of the English vital statistics for 1910 which by chance had been given to his clinic and made a graph of the deaths from tuberculosis by age and sex. He found from these figures also that the curve for boys rises several years later than it does for girls. In this way he became interested in vital statistics and made an extensive study of the excellent series for England and Wales. He hoped to cover the whole subject but the part on malignant tumors was the only part complete at the time of his death and this, put through the press by his friend, Dr. Herbert Orel, forms the present volume.

Part one consists of large numbers of curves showing deaths from tumors according to age, sex, and site of tumor. He finds that these graphs are of different types, that is, for example, the age incidence of tumors differs for different parts of the body or for different organs, and by bringing the graphs of similar types together into groups he shows the reactivity or susceptibility of the parts of the body for tumors according to age or as he calls it the

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*Ergebnisse der medizinischen Strahlenforschung. (Results of the investigations of medical radiation including x-ray diagnosis, and the therapeutic use of roentgen rays, radium and light.) Vol. III, 1928; IV, 1930; V, 1931. Georg Thieme, Leipzig.

**Ueber die Akute und Chronische Gelbe Leberatrophie mit Besonderer Berücksichtigung ihres Epidemischen Auftratens in Schweden in Jahre 1927. (Acute and Chronic Yellow Atrophy of the Liver With Especial Reference to Its Appearance in Epidemic Form in Sweden in 1927.) By Prof. Dr. Hilding Bergstrand. Published by Georg Thieme, Leipzig, 1930.

†Allergie des Lebensalters—die bösartigen Geschwülste. The Allergy of Age—the Malignant Tumors. By Dr. Clemens Pirquet. Published by Georg Thieme, Leipzig, 1930.

allergy of age. In this way he makes what he considers natural groups of tumors as follows: those of the abdomen, thorax, mouth, male sex organs, female sex organs, skin, and other organs.

Human Biology and Racial Welfare*

THIS is yet another volume in which Edmund Cowdry demonstrates his abilities as an editor and impresario. First, he has good ideas concerning subjects that would be of special interest, and second, he has the knack of persuading men who are peers in their respective fields to collaborate with him. As the title would indicate, this is a volume of no mean ambition. Indeed, it is a compilation of the frontiers of thought and investigation in the social sciences and in the study of man and his environment.

The start is breath-taking enough, dealing as it does with what we know of the whole universe, particularly of the possibilities of life in the universe. Following this are several chapters on the origin of man and evolution—individual, mental, and social. Then, comes a discussion of man himself as a physiological unit, built up from the cells or vital units, through cell aggregates; and the importance of the vascular system, later of the nervous system, and on through the more highly complex discussion of sex integration.

Part Four deals with the effects upon man of his environment and Part Five ventures some prophecies as to the future. The list of contributors is breath-taking and the scope of the discussion is almost truly universal as it relates to man.

This is a monumental work, the kind that a really intellectual man likes to have near him for piecemeal reading, with the assurance that all that he can absorb therefrom is authoritative and will keep him conversant with the most advanced thought of the day in the study of mankind.

You have seen the advertisement of the bashful young man who never had anything to say at social gatherings, but who after taking the Benjamin Franklin home correspondence course in French astounded his friends by his subsequent ability to lead the field in any intellectual conversation. The reviewer ventures to say that anyone who can readily assimilate all that is in this volume would be able to acquit himself regally thereafter in any really intellectual drawing room levee.

Not all of the contributors have been universally successful in acquitting themselves equally well in grading down the tempo of their discussion to the speed of the average reader, not specialists in their own subjects, and as a consequence parts are rather hard reading, but the majority have achieved this admirably.

American Physicians and Surgeons†

THIS book is a really laudable effort to establish a reliable Who's Who in American Medicine. Directories of American physicians and surgeons have appeared in the past but a cursory examination almost always reveals that the price of having one's name appear therein is the purchasing of a copy of the directory.

This volume, issued by the publishers of "The American Bar" which has been a recognized Who's Who in the legal profession for several years, has been developed strictly on a who's who basis. The selection of names has been based on membership in special societies and direct reference to the prominent physicians of each community.

As is inevitable in a first edition, the names of a number of outstanding men in different communities have been left out. But it is better to have errors of omission than of commission. In those cities with which the reviewer is well acquainted there have been practically

*Human Biology and Racial Welfare. 28 Authors. Edited by Edmund V. Cowdry, Professor of Cytology, Washington University, St. Louis. With an introduction by Edwin R. Embree. Illustrated. Cloth. Pages 612. Paul B. Hoeber, Inc. New York. 1930.

†American Physicians and Surgeons. A Biographical Directory of Practicing Members of the Medical Profession in the United States and Canada. Including supplements in which are listed and classified the leading hospitals, sanitariums and health resorts of both countries. Prepared by James Clark Fifield, Editor of The American Bar. Leather. Pages 1737. The Midwest Company. Minneapolis. 1931.

no names included who did not deserve the recognition. The others who should be in can easily be added in the next edition.

Of course there are many who did not fill out the biographical data requested before the publication of the volume. Their names however appear with as much information as the editors could cull from the directory of the American Medical Association and the list of the special societies.

The price of the volume is high but we presume that it must be so in a first venture of this sort in which the outcome is uncertain.

We hope that the editors will succeed, will adhere to their high standard which they have set for themselves, and will be able before long to reduce the price of the volume.

Symptoms of Visceral Disease*

OCCASIONALLY a medical prophet, far ahead of his times, will conceive and develop a new interpretation of disease or of certain diseases. He may tell his story repeatedly to an unheeding multitude, unwilling or unequipped to grasp the significance of the story. If he is so unfortunate as to pass on before the average doctor has gained an adequate comprehension of his theory, all of his labors will be lost for a time until some new thinker, possibly many years later, will rediscover the idea. Such a man was Sir James MacKenzie and such an idea was his principle of the reflex arc.

Why is it that the average doctor shuns all invitation to any profound study of organic neurologic reflexes and responses? Is it because as a rule he is poorly trained in neurology and prefers to leave the subject for a specialist? Scarcely any field of medicine is more nearly a true science than that of organic neurology and neurologic localization, and diagnosis is a very simple thing provided one really knows the anatomy of the central nervous system. All that is required is a little real study.

The trained neurologist is too often so occupied with his studies of organic disease of the central nervous system and peripheral nerves, that he has scant time to give to the neurologic facors in functional visceral reflexes.

It is the internist who should be primarily interested in the latter, for as a rule these reflexes are manifested not so much as disease of the nervous system, but more as disease in the internal organs which only occasionally produces functional neurologic manifestations.

While MacKenzie was unfortunate in that he did not live to see a general acceptance of his principle of the reflex arc, he was fortunate in having a disciple able to comprehend his teachings and to carry on. The volume by Dr. Pottenger under review is however, not a mere continuation of MacKenzie's teachings. It is a summary of a life study by the author into which he has incorporated much of MacKenzie's teachings and in which he has correlated these with his own entirely original, and different in many respects, concepts of the functional neurologic reaction in visceral disease.

Years ago Dr. Pottenger became enthused over a study of the autonomic nervous system with its two antagonistic subsystems, and from his investigations he rapidly popularized the concept of the function of the vegetative nervous system. While his first interest was in its action as related to pulmonary disease he has extended this to visceral disease in general.

The volume does not lend itself well to abstraction, but we may state without hesitation that it is the most comprehensive and intelligible of the available treatises on the function of the vegetative nervous system in its relation to the remainder of the nervous system and to organic visceral disease as applied to clinical medicine. The fact that it has already reached its fourth edition would indicate that there are many doctors who do not possess the illusion that neurologic studies are too dry or too deep.

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*Symptoms of Visceral Disease. A Study of the Vegetative Nervous System in its Relationship to Clinical Medicine. By Francis Marion Pottenger, A.M., M.D., LL.D., F.A.C.P., Medical Director, Pottenger Sanatorium for Diseases of the Lungs and Throat, Monrovia, California. Fourth Edition. With 87 text illustrations and 10 color plates. Cloth. Pages 426. The C. V. Mosby Company, St. Louis, 1930.

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EDITORIAL

Malignancy in Radioactive Persons

Radium poisoning as a new and exceedingly dangerous occupational disease was first brought to light by the studies of Martland and his associates,[1] in 1925 as a result of investigations conducted upon individuals engaged in the manufacture of luminous watch dials.

From these studies it was shown that radioactive substances entering the body by ingestion, absorption through the skin, or inhalation were eventually stored as an insoluble sulphate, in particulate or colloidal form, in the main organs of the reticuloendothelial system and especially in the long bones from which latter site in particular they continued to emit their characteristic emanations.

It has been demonstrated also that as little as one one-hundred-thousandth of a gram of radioactive substance so deposited continues to emit alpha emanations at an incredible speed and in innumerable numbers for indefinite periods of time so that it has been estimated, for example, that assuming such a deposit to have occurred in the body in 1925, the skeleton will still be emit-

ting 185,000 alpha particles per second at a speed of 18,000 miles per second in the year 3491 A. D.

As the alpha particles are probably the most potent and obstructive agents known to science, the importance of this industrial hazard becomes at once apparent.

As a result of this constant and continual radiation from the deposits in the bones, the bone marrow, and the blood-forming organs, periods of irritative stimulation and overstimulation are followed by periods of exhaustion, the symptomatic expressions of which are seen in necroses of the jaw and in the development of a leucopenic anemia of regenerative type, resistant to all means of treatment and ultimately, as well as often rapidly, fatal.

The bone necroses resulted from the occurrence of an intense osteitis and when, as in the jaw, a superimposed bacterial invasion occurred, necrosis was a natural aftermath.

These were the early symptoms, and it has remained for continued study and observation the results of which are now reported by Martland[2] to establish the fact that a late result of the deposit of radioactive substances in the human body is the inevitable development of rapidly growing embryonal or anaplastic osteogenic sarcomas.

Six deaths are now reported from this cause and several other cases are on record in which death has not as yet occurred but in which a fatal outcome is inevitable.

The sequence of events in the production of these terrible sequelae is believed by Martland to be, first, a hyperplastic marrow irritation, succeeded by a period of replacement fibrosis which in its initiation is very cellular and as a product of which osteogenic sarcoma occurs.

For the first time in the history of medicine the story of the effect of the internal bombardment of human tissues by the alpha particle has been recorded, an imposing list of lesions: irritative hyperplasia and compensatory stimulation of the bone marrow of a very primitive type; leucopenias, mild anemias of the pseudoaplastic type; fatal anemias of the regenerative or megaloblastic type with leucopenias approaching an agranulocytosis, but with no evidence of hemolysis and rarely marked hemorrhagic tendencies; replacement fibrosis with production of a radiation osteitis; necrosis of the jaw due to a superimposed infection upon a radiation osteitis; crippling and deforming bone lesions due to healing radiation osteitis; with coxa vara, deformities of the spine, spontaneous fractures, etc.; a packing of the bone marrow with primitive stem cells resembling leucemoid state; the possibility of the development of myeloid leucemias and even multiple myelomas, and finally osteogenic sarcomas.

Martland also very pertinently emphasizes the relation of his findings to the many radioactive waters, emanators, activators and so on which are offered for sale to a credulous public.

Even though their content of radioactive substances may be so slight as to render any good effects from their use purely psychic, the studies above summarized clearly show that extensive lesions of serious character follow

the implantation in the body of incredibly small amounts of radioactive substances. Even less than one-half a microgram may be dangerous in Martland's opinion.

That the conception of radioactive waters as a dangerous source of deposit of such substances in the body is neither extreme nor fallacious is shown by the fact that persons ingesting them over a period of one or two years have been shown to have radioactive deposits in their bodies, demonstrated both by electrometric tests, and also by the occurrence of bone necrosis.

The study, and the reports upon which it is based and from which it has developed, are of absorbing interest, not only as recording the story of a new and terrible industrial hazard; not merely as an outstanding scientific achievement; but also as suggesting new avenues for the experimental study of malignancy.

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—*R. A. K.*